VISUAL AND RETINAL CONTROL OF EYE GROWTH AND REFRACTION

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Under the requirements of PhD regulation 9.2, the above candidate was examined orally by the Faculty. The members of the panel set up for this examination recommend that the thesis be accepted by the University and forwarded to the appointed Committee for examination

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Under the requirements of PhD regulation 9.15, it is hereby certified that the thesis of the above-named candidate has been examined. I recommend on behalf of the Examination Committee that the thesis be accepted in fulfilment of the conditions for the award of the degree of Doctor of Philosophy.

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The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Katrina L. Schmid
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ABSTRACT

In contrast to previous investigations of myopia development in humans that have primarily examined the effectiveness of therapies, the present thesis examines the visual factors underlying the emmetropization process and the formation of form-deprivation myopia. The chick has been used as a model in this research. The experiments have been designed on the premise that emmetropization is a vision-dependent phenomenon, with information provided by the visual image guiding the growth of the eye.

Initially the effects of physiological variants were studied. The results showed that normal ocular growth rates are both breed- and sex-dependent in the chick, and also that different breeds of chicks differ in both their susceptibility to form-deprivation myopia and in the speed of their recovery from form-deprivaiton myopia. This result has important implications with respect to interpretations and comparisons of studies using differenct breeds and/or sexes of chicks.

Interrupting deprivation with even a very short period of normal vision had a marked effect on deprivation-induced myopia. Neutralization of a "myopic defocus signal" was found to occur in less than 20 minutes. Periods of normal vision from 20 min to 60 min were equally effective at reducing the high myopia and suggest that defocus is not sampled continually; most of the recovery appeared to be due to choroidal expansion. The effectiveness of normal vision in guiding emmetropization was found to be independent of the timing of the experience. Periods of normal vision given in the morning, i.e. at the start of the light cycle, were as effective as those given in the afternoon, i.e. at the end of the light cycle, in preventing occlusion-induced myopia.

Low powered spectacle lenses, i.e. +1 D and –1 D were used to determine the sensitivity of the emmetropization process to defocus. Compensation for the lenses occurred even though the depth-of-focus of the chick eye was estimated to be between ±0.75 D and ±2 D and the defocus may have been below the detection threshold of the retina. Similarly compensation for larger induced refractive errors, i.e. +10 D and –10 D occurred. Hyperopia in response to +10 D lens wear occurred very rapidly and some hyperopia was seen even when lens wear was interrupted by long periods of normal vision. In contrast, the response to

-10 D lenses was much slower and myopia was only observed if the lens was applied continuously. As a possible explanation for the differential response to different types of lenses it was suggested that accommodation would differentially affect the degree of "image blur", with large degrees of defocus for positive lenses and effectively none for the negative lenses. The experiment was thus repeated following ciliary nerve section to eliminate accommodation as a factor. The results found with ciliary nerve section were similar to those obtained without and thus accommodation does not explain the nonlinearity of effect. An alternative model involving the speed of physiological growth responses was suggested. The similarity of the effect of periods of normal vision on form-deprivation myopia and lens-induced myopia suggests that similar emmetropizing growth signals are experienced during the period of normal vision regardless of the method of myopia production.

To investigate further the role of accommodation in emmetropization, compensation to negative spectacle and hard contact lenses were compared. Hard contact lenses were presumed to decrease the gain of the accommodation system by eliminating the refractive effects of corneal accommodation; thus for any given accommodative "effort" the refractive effect would be lower. Emmetropization occurred in response to both types of lenses, again suggesting that accommodation has a limited role in the emmetropization system. Other likely defocus signals were thus investigated.

The chick eye was found to possess 3.7 D of longitudinal chromatic aberration, measured by chromoretinoscopy. Longitudinal chromatic aberration can potentially provide information about defocus and hence its role in emmetropization was studied by rearing chicks under monochromatic light. It was found that adequate information was provided by monochromatic light to guide emmetropization in the chick, whether the monochromatic light was from the extremes (red and blue light), or the centre (yellow light), of the visible spectrum. However, it was also found that emmetropization to the refractive difference present in different coloured lights does not occur. The results of this study were supported by an experiment using an interrupted-occlusion paradigm. It was assummed that during the period of "normal vision", if the information provided was adequate, that defocus cues would be detected and the effect of occlusion reduced. Chicks were monocularly occluded and the occlusion interrupted by a brief exposure to "normal vision" per

day under monochromatic light. Prevention of myopia, i.e. emmetropizing growth, was observed. The results of both of these studies suggest the presence of non-chromatic cue or cues to defocus.

The interrupted-occlusion paradigm was likewise used to investigate possible non-chromatic cues to defocus. During the period of occluder removal stimuli of either restricted contrast or restricted spatial frequency information were presented. Restricted contrast environments were found to be as effective as normal vision at reducing the magnitude of occlusion-induced myopia. The data indicated that a varied contrast environment is not required for emmetropization but suggest the presence of a contrast threshold, less than 4% for the chick, below which emmetropization becomes inaccurate. Emmetropization was spatial-frequency-dependent; the ability of restricted spatial frequency environments to reduce occlusion-induced myopia varied with the spatial frequency presented. The data further indicated that mid-spatial frequencies are required for emmetropization. The results suggest that there are additional as yet unknown non-chromatic cue for defocus.

The main findings of this thesis show that in the chick:

i) myopic defocus is detected within 20 min,
ii) emmetropization to refractive defocus of opposite signs is highly non-linear and differentially affected by normal vision,
iii) emmetropization occurs under monochromatic light from the centre and extremes of the visible spectrum,
iv) there is at least one non-chromatic visual cue involved in emmetropization,
v) a varied contrast environment is not required for emmetropization,
vi) emmetropization is spatial frequency dependent,
vii) intermediate spatial frequencies appear to be required for emmetropization.

A preliminary model is proposed for the refractive error detector of the visual system.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CHAPTER 1

INTRODUCTION

If the eye is too long or too short for its refractive power a significant refractive error, i.e. myopia or hyperopia, will be present. Yet in most cases the position of the retina lies at the exact image plane for distant objects, i.e the eye is emmetropic. Hyperopic and myopic defocus appear to provide different retinal image signals which are translated into differential growth signals to guide emmetropization. Evidence for this comes indirectly from human data but largely from experiments involving chicks and other animals. An unresolved issue, that is taken up in this thesis, is what visual signal or signals are guiding the emmetropization process and why is it that this process goes awry causing refractive errors to develop. As background to this issue, research investigating the emmetropization process in both humans and animals is reviewed. In particular, human refractive error development, anomalous eye growth patterns in the chick and other animals and visual information of possible importance to the emmetropization system are discussed.

1.1. Human Refractive Development and Myopia

1.1.1. Emmetropization: a Vision Dependent Phenomenon

At birth, humans have widely distributed refractive errors with hyperopic refractions being most common. During development, the magnitude of refractive errors diminish, so that by 1 year of age most eyes are emmetropic and few are ametropic. This process is called emmetropization (Cook and Glasscock, 1951; reviewed in Banks, 1980 and Hirsch and Weymouth, 1991). There has been much debate over whether this process is an active one involving a closed-loop system guided by visual feedback (van Alphen, 1961; reviewed in Medina and Fariza, 1993) or a passive one, i.e. as the eye increases in size refractive errors appear to decrease due to a better match between the focal length and physical length of the eye irrespective of the influence of vision (Hofstetter, 1967; Edwards, 1992).

The active visual feedback hypothesis is strongly supported by human data, which has shown that emmetropization can be disrupted by environmental manipulation. When normal vision is disturbed through ocular pathology, the emmetropization process is disrupted. Deprivation of high quality pattern vision in human infants, e.g. due to ptosis (O'Leary and Millodot, 1979), hemangiomas (Robb, 1977), neonatal eyelid closure (Hoyt *et al.*, 1981) or retinopathy of prematurity (Rabin *et al.*, 1981), has been shown to result in axial elongation and high myopia. More generally, low vision children who are deprived of normal visual experiences exhibit an increased incidence of both high hyperopic and high myopic refractive errors (Nathan *et al.*, 1985; Wildsoet and Lovie-Kitchin, 1989).

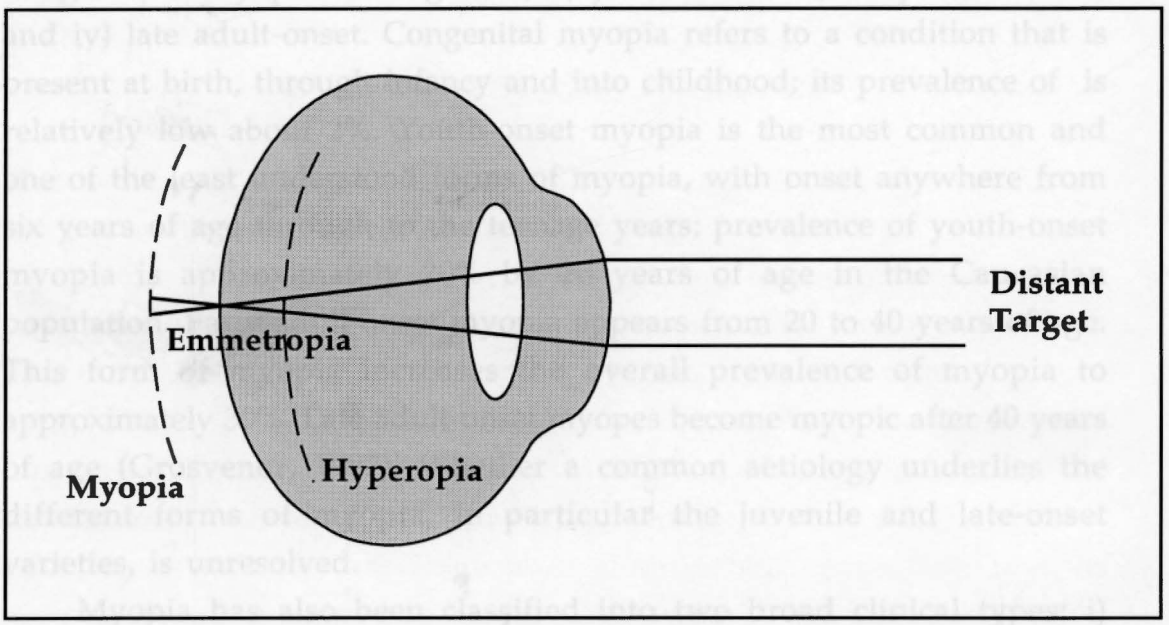


Figure 1.1. Light rays from distant targets come to focus at the retina of an emmetropic eye. For a myopic eye the rays are focussed in front of the retina, and for a hyperopic eye the best focus lies behind the retinal surface.

Myopia affects over 30% of the population and can lead to severe visual impairment (Sperduto *et al.*, 1983; reviewed in Curtin, 1985). The optical basis of myopia is well understood. Myopia occurs as a consequence of an eye being relatively too long for its optical power, resulting in images of distant objects being focussed in front of the retina (Fig. 1.1). Although high degrees of ametropia can be explained by heredity or pathological

causes, smaller refractive errors are more difficult to explain. Conjecture still exists as to the cause of myopia in the absence of obvious visual disturbance and there is much debate as to the best form of preventive treatment.

1.1.2. Classification and Magnitude of the Problem

There are undoubtedly several types of myopia of varying severity and aetiology. In an attempt to understand myopia and its development, many researchers have tried to classify myopia into discrete categories. Grosvenor (1987) reviewed existing classifications and proposed a new system based on age-related prevalence and age of onset. He proposed four categories of myopia: i) congenital, ii) youth-onset, iii) early adult-onset, and iv) late adult-onset. Congenital myopia refers to a condition that is present at birth, through infancy and into childhood; its prevalence of is relatively low about 2%. Youth-onset myopia is the most common and one of the least understood forms of myopia, with onset anywhere from six years of age through to the teenage years; prevalence of youth-onset myopia is approximately 20% by 20 years of age in the Caucasian population. Early adult-onset myopia appears from 20 to 40 years of age. This form of myopia increases the overall prevalence of myopia to approximately 30%. Late adult-onset myopes become myopic after 40 years of age (Grosvenor, 1987). Whether a common aetiology underlies the different forms of myopia, in particular the juvenile and late-onset varieties, is unresolved.

Myopia has also been classified into two broad clinical types: i) physiologic myopia, and ii) pathologic myopia (Curtin, 1979). Physiologic myopia is an optical condition of the eye in which a combination of refractive components of normal dimensions renders the eye myopic. An increase in curvature of the surfaces of the cornea or lens, or an increased axial diameter of the eye attained by normal growth, may be underlying factors, each being capable of producing myopia unless proportional compensatory changes are present in the other components. In contrast, pathologic myopia is a direct consequence of an abnormally dimensioned ocular component. In the strict sense, this is limited to myopia associated with an abnormal lengthening of the eye accompanied by staphyloma formation.

If myopia only caused a significant dependence upon optical correction, it would be a problem of major dimensions, affecting 25% to

30% of young adults. However, of greater importance is the severe reduction in corrected vision that is associated with the pathologic form of myopia (reviewed in Curtin, 1979) and the serious ocular complications that can occur, even from relatively low myopia. There is a higher incidence of retinal detachment, glaucoma, cataracts and chorioretinal degeneration in highly myopic eyes compared to near emmetropic ones (reviewed in Curtin, 1970; Curtin, 1985). The National Eye Institute found that myopia was the eighth most frequent cause of severe visual impairment and the seventh most frequent cause of legal blindness in the United States (determined 1976, cited in Curtin, 1979). Visual impairment due to myopia may be even higher than that reported, as the blindness of a myopic eye, e.g. due to retinal detachment, is often reported as due to retinal rather than myopic causes (Curtin, 1985).

1.1.3. Myopia Aetiology and Therapy

Human myopia research has focussed on developing strategies to prevent myopia or at least to slow its progression. Reasons for myopia progression have been much sought and analysis of age, environment, heredity, intelligence, nutrition, ocular dimensions, personality, race and sex have been conducted (for reviews see Borish, 1977; Curtin, 1985; Grosvenor and Flom, 1991). However environmental influences have been given the most attention. It is now known that these play some role in the development of myopia and that the degree of myopia is not determined solely by genetic factors. The role of environment versus heredity has been extensively reviewed by Young (1975), Goss (1982), Goss *et al.* (1988), Curtin, (1985) and more recently by Bear (1991) who made a strong case, from a geneticist's point of view, for "vision activity" influencing the development of myopia. The amount of nearwork appears to account for more of the similarity in refractive errors of family members than does genetics (Bear, 1991). It would appear that the issue today is not whether ametropia is genetically or environmentally determined, but rather what the relative roles of genetics and environment are. Thus it would appear that myopia involves a complex interaction of genetic and environmental factors.

Prolonged nearwork as a cause of myopia

Much speculation exists regarding the relationship between myopia and intensive nearwork. A long held belief is that excessive nearwork causes, or is a major contributing factor in the development of myopia. As early as 1879 Javal investigated why people became fatigued when they read for extended periods, but constant distance viewing produced no symptoms. Javal stated that "prolonged use of the eyes on adjacent objects as the cause of fatigue is so universally recognized that it is not doubted by anyone" and it is "through habitual accommodative effort" that he explained the fatigue of writers and their resultant myopia (Javal, 1879, reprinted and translated by Ciuffreda and Bassil, 1990).

Evidence for nearwork causing myopia is the positive relationship between myopic progression and time spent reading (Pärssinen *et al.*, 1989). Low levels of education are associated with low frequencies of myopia and high levels with high frequencies of myopia (Richler and Bear, 1979; reviewed by Curtin, 1985). Angle and Wissmann (1978) also linked education with myopia appearance and progression. They derived a progression rate of 0.22 D per academic year for students in their study, in the 12 to 17 year age group. Epidemiological studies on populations in Alaska (Young *et al.*, 1969), Greenland (Alsbirk, 1977), and Newfoundland (Richler and Bear, 1980), have reported increased prevalence of myopia in present generations compared with past generations. Young *et al.*, (1969) suggested that the most likely cause of the "epidemic of myopia" in this Eskimo population was the introduction of Western-style education and hence increased nearwork. Also, an association between myopia and occupations with unusually high nearwork demands does exist (e.g. law students, Zadnik and Mutti, 1987; microscopists, Adams and McBrien, 1992; medical students, Midelfart *et al.*, 1992; reviewed by Curtin, 1985).

Many different theories have been proposed for how near vision tasks could trigger or cause myopia. Theories based on mechanical stress being exerted on the posterior sclera by the extraocular oblique muscles during accommodation (reviewed by Greene, 1991), increased vitreous chamber pressure during accommodation causing scleral stretching and myopia (reviewed by Young and Leary, 1991), sustained near work producing adaptations in accommodation and vergence that in the long term permanently alter refractive state (reviewed by Schor, 1991) and increased ciliary tonus produced from near work (reviewed by Owens, 1991) have been suggested.

Myopia treatment based on relaxing accommodation

Most myopia therapies aim to slow myopia progression by reducing or relaxing accommodative activity based on the aforementioned premise that excessive accommodation causes myopia. Numerous therapies rely on this theory; bifocal spectacles, under correction, cycloplegic drugs and biofeedback training have been instigated as myopia "treatments".

Should myopes be prescribed bifocal lenses or simply single vision spectacles? Reports on the success of bifocal therapy show considerable disagreement. Goss (1986) determined that the rate of myopia progression was slowed by the wearing of bifocal spectacles for those childhood myopes with nearpoint esophoria or a high-plus cross-cylinder-net; the rate of progression was not significantly different for all other myopes. However, a study by Grosvenor (1987) did not confirm these findings; no difference in myopia progression for bifocal compared with single vision lens wearing groups was observed. In yet another study (Pärssinen *et al.*, 1989) myopic progression among schoolchildren appeared unaffected by bifocal wear or the practice of avoiding spectacle use when reading. In addition to the equivocal results with bifocals the reverse treatment, spectacle overcorrection of myopia, which theoretically should increase progression rates did not significantly alter progression (Goss, 1984; Rutstein *et al.*, 1989). As a reason for the poor results of bifocal treatment, Grosvenor (1987) suggested that once a child was myopic and the sclera stretched and thinned, the sclera would continue to stretch even if excessive accommodation was prevented.

Cycloplegic agents, such as atropine, have been used to relax accommodation and thus control myopia. Daily administration of 1% atropine was found to inhibit the progression of youth-onset myopia in treated eyes (Bedrossian, 1971; Brodstein *et al.*, 1984). It was suggested that this decrease in progression was evidence for accommodation as a causative factor in human myopia development, although, as atropine has effects other than blocking accommodation, there are other explanations for this decrease.

Biofeedback training has been described as an alternative way to reduce myopia and/or slow progression by relaxing accommodation. During therapy, an auditory signal is used to indicate to the myopic patient when they are relaxing their accommodation and that situation is then aimed for during training. The results from this technique are also

controversial, with improvements in acuity rather than reductions in myopia often reported (Trachtman, 1987), and questions as to whether the improvements in acuity simply represent a learning effect have been raised (Gallaway *et al.*, 1987).

To date there are only conflicting views as to the effectiveness of various treatment regimens designed to slow myopia progression, with results taken together presenting negative or inconsistent pictures (Goss, 1982; Grosvenor, 1987; Pärssinen *et al.*, 1989; reviewed by Grosvenor, 1991). Accommodative theories for myopia predict that myopia progression should halt or slow when using bifocals or reading without spectacles. However, this does not routinely occur. Although myopic shifts, in both the tonic position of accommodation (Ebenholtz, 1983; Schor, 1984) and the far-point (Rosenfield *et al.*, 1992) have been observed after sustained nearwork, other studies do not support the theory of accommodative hysteresis as a causal factor for nearwork myopia (Fisher *et al.*, 1987) and van Alphen has suggested that increased accommodative tonus may actually reduce rather than increase stress upon the sclera by modulating choroidal tension (van Alphen, 1986). Thus, the research summarized above does not convincingly support accommodative theories.

Other myopia treatments

Other non-accommodative myopia treatments include contact lenses and refractive surgery. The effectiveness of hard contact lenses in controlling myopia progression is controversial; corneal flattening, i.e. orthokeratological effects, seems to account for most of the reported decrease (reviewed in Curtin, 1985; Grosvenor and Goss, 1988). Hard contact lenses fitted flatter than the flattest corneal meridian cause corneal flattening. Recently, Grosvenor *et al.* (1991a) have suggested that contact lenses may also have an effect on the axial elongation of the eye, although, strangely, these changes do not seem to persist after contact lens wear is ceased (Grosvenor *et al.*, 1991b). It has been suggested that the slowed progression with contact lens wear may be due to an improvement in retinal image quality when contact lenses rather than spectacles are worn. However, there is no associated benefit of soft contact lens wear on myopia progression (Andreo, 1990).

Radial keratotomy and photorefractive keratectomy (eximer laser surgery) have become increasingly popular as myopia therapies (reviewed in Grosvenor, 1991 and Lakkis and Brennan, 1993). While significant

reductions in refractive errors do occur with the treatment, other postsurgical effects, such as scarring and glare for radial keratotomy and stromal haze and opacification for photorefractive keratectomy, are of concern. Long-term refractive stability is unlikely for some patients.

1.1.4. Alternative Models of Nearwork Myopia

Although the classical theory has been that prolonged, chronic accommodation while reading leads to myopia however there are other peculiarities of nearwork. An alternative theory is that poor image quality (Wallman *et al.*, 1987) or blur promotes myopia, i.e. myopia is an adaptive response to nearwork rather than an effect of excessive accommodation. This could explain the poor results obtained with myopia therapies aimed at slowing myopia progression by reducing accommodative demand.

There are many ways that retinal blur can arise. To obtain clear vision while reading, the accommodative response must approximate and be maintained at the level of the accommodative stimulus. Under typical conditions the accommodative response is approximately 2.25 D for an accommodative stimulus of 2.50 D (Ward, 1987). While a small lag of accommodation usually occurs, clear near imagery is assumed due to the eye's depth-of-focus. However, if the lag of accommodation is greater than normal, the retinal image quality may be affected and myopia may result from a similar mechanism as that in form-deprivation myopia (Fig. 1.2). Charman (1983) has suggested that refractive correction and accommodation should be accurate to $\leq \pm$ 0.25 D for a noticeable deterioration in retinal image quality to be avoided. A reduced ability to accommodate over lens-induced blur has been demonstrated in myopic subjects (Gwiazda *et al.*, 1993) and it has been suggested that this accommodative dysfunction may underlie myopia development. Optical defocus, e.g. due to accommodative insufficiency, may induce an artificial hyperopia during nearwork, the ocular images focussed behind the retina initiating an increase in axial growth so as to move the retina in the direction of the focussed image (Fig. 1.3).

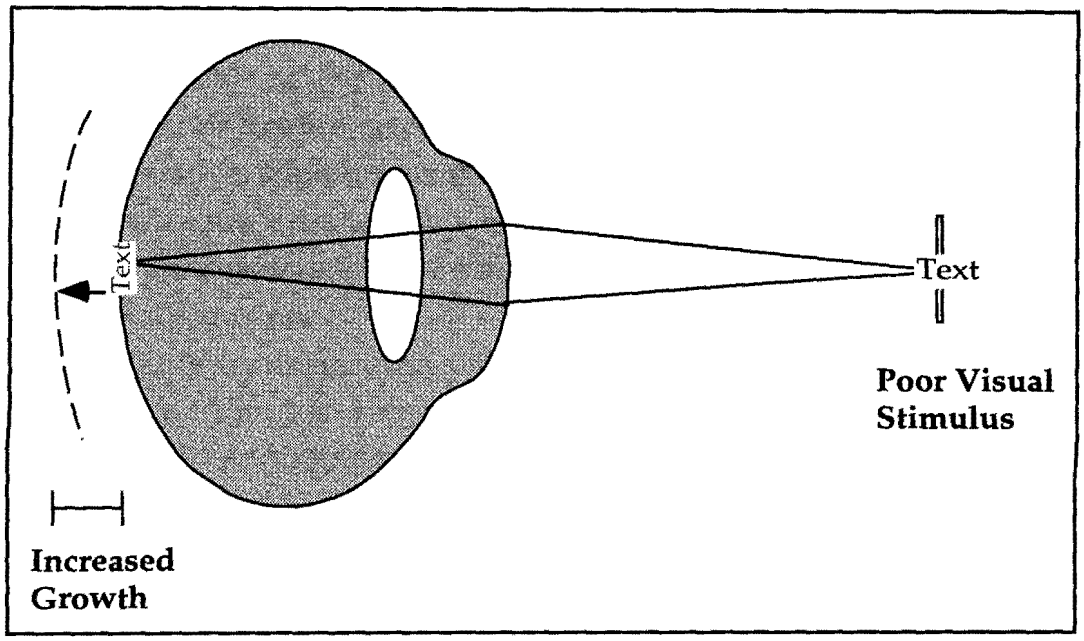


Figure 1.2. Model for development of myopia due to inadequate visual stimulation while performing nearwork. Retinal image quality may be inadequate in the presence of slight blur or a poor quality visual stimulus, resulting in myopia due to a similar mechanism to that in form-deprivation myopia.

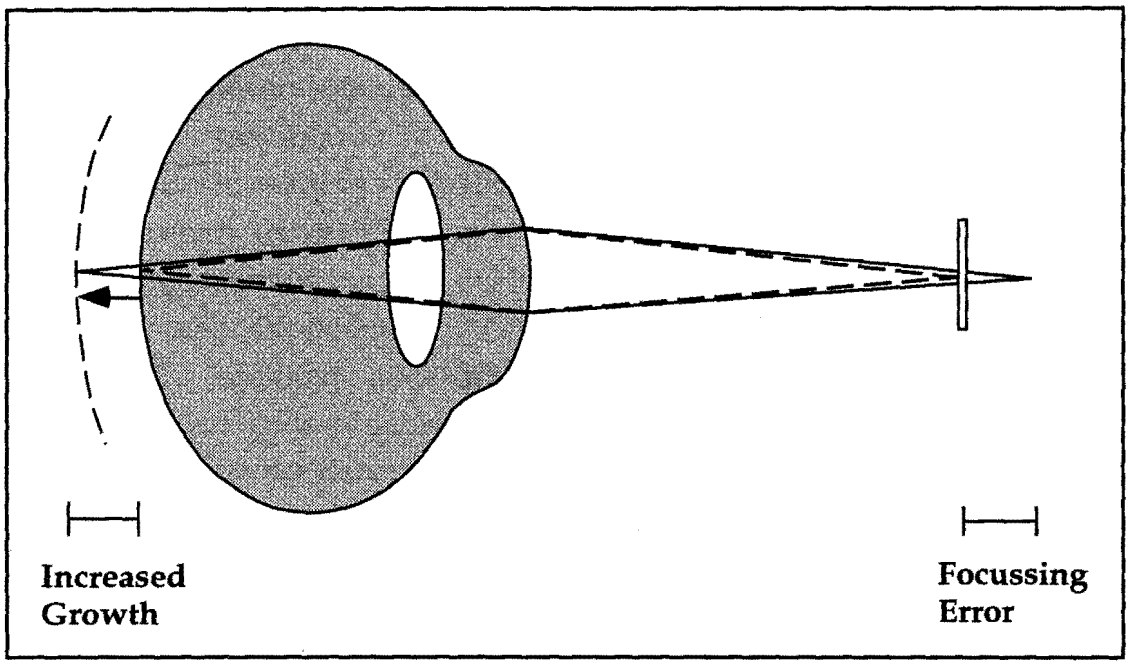


Figure 1.3. Model for the development of myopia due focussing errors while reading. Optical defocus, e.g. due to accommodative insufficiency or an excessive accommodative lag, may induce an artificial hyperopia, the ocular images focussed behind the retina initiating an increase in axial growth so as to move the retina in the direction of the focussed image

Although accommodation is dominated by the central retina (Fincham, 1951) it has also been demonstrated that peripheral visual stimuli can influence accommodative level (Hennessy, 1975). As the accommodative stimulus will be less in the periphery due to a slightly greater working distance, this may contribute to the accommodative lag and myopia development. Alternately, blurred peripheral vision may be a stimulus for myopia.

Reading material, even with accurate focussing, may still provide a poor visual stimulus due to the limited variability of the material, i.e. small, black writing on a white background (Wallman *et al.*, 1987). Similarly, letters or numbers displayed on visual display terminals are often of poor legibility and may provide inadequate focus cues (reviewed in Lovasik and Kergoat, 1988). Visual display workers often report visual symptoms such as transient blur and visual fatigue (Collins *et al.*, 1991; reviewed in Dainoff, 1982) and this blur may lead to a deprivation myopia. There is also thought to be a high rate of myopia progression in visual display users (Tokoro, 1988), although a recent study did not support this finding (Yeow and Taylor, 1991).

A highly speculative hypothesis is that the poor visual stimulus results from the nature of the reading process itself. There are constant saccadic eye movements during reading, practised readers move their eyes on average every quarter of a second (reviewed in Carver, 1990), and as children gain in reading skill, their eye fixations become shorter and their eye movements longer (Zola *et al.*, 1992). Visual information is absorbed only during fixation pauses; during saccades, there is a deterioration in target detection (Volkmann, 1962). The image during a saccade, although perceptually inhibited, may appear blurred and a deprivation situation result. However this proposal is unlikely. Given that fixation pauses are long enough for the detection of complex visual information, they should similarly be long enough for the emmetropization mechanism.

1.2. The Chick: an Animal Model for Eye Growth

Many of the problems inherent in research involving humans are avoided by the use of an appropriate animal model. Research involving animals, most commonly chickens, has greatly added to the understanding of the processes involved in eye growth regulation and refractive error development. It must be mentioned at the outset that there are some fundamental differences between the avian and primate visual systems: as opposed to the primate the avian retina contains a pecten, visual streak

and up to two fovea (Ehrlich, 1981), the iris and ciliary muscles consist of skeletal muscle, both lenticular and corneal accommodation are possible (Troilo and Wallman, 1987), the sclera has fibrous and cartilaginous layers and there is total decussation of the primary visual pathway (Pumphrey, 1961; Walls, 1967).

The chick provides a useful model in that its eyes are fast growing and results can be obtained rapidly. While the appropriateness of the chick as a model has recently been questioned (Andison *et al.*, 1992), the suggested avian alternative, the American kestrel is a poor alternative. At hatch the kestrel is highly myopic, dependent on its parents for food and has partially closed eyes (Andison *et al.*, 1992). In comparison, both primates and chicks tend to emmetropize from a hyperopic starting point. In addition, young chicks peck fine food grains soon after hatch suggesting that chicks are the more visual of the two species and thus more similar to primates in this respect also.

In chicks, form-deprivation, dark rearing, dim light and continuous light studies have provided evidence that emmetropization requires normal vision and particular lighting conditions during rearing.

1.2.1. Normal and Anomalous Eye Growth in Chick

Normal growth

At hatching chicks are normally slightly hyperopic, over a period of 8 weeks this hyperopia is reduced and emmetropia results (Wallman *et al.*, 1981). Both the magnitude of the refractive errors and the spread of refractions decrease during this time. It has been suggested that eye growth occurs in two stages: i) embryonic or pre-hatch eye growth which ensures the eye is of a certain gross size and shape, and ii) emmetropic or post-hatch eye growth which fine-tunes the refractive components of the eye (Wallman *et al.*, 1981). Oishi and Murakami have shown that only very short periods of vision are required, normal growth occurring in chicks exposed to light for only 4 hrs/day (Oishi and Murakami, 1985).

Form-deprivation myopia

In chicks, large myopic refractive errors develop rapidly in response to deprivation of form vision, as produced by either lid suture or translucent occluders (goggles). This phenomenon was first reported by Wallman *et al.* (1978b) who showed that vision-dependent mechanisms underlie occlusion-induced changes in refraction and eye growth. Neither lid suture nor occlusion prevent light reaching the retina (Yinon, 1984), but such

techniques cause significant retinal image degradation. It is this reduction in image quality that has been postulated as the cause of myopia rather than mechanical or thermal disturbances to the eye (reviewed in Wallman, 1991). Opaque, black occluders have also been used to induce myopia in chicks (Sivak *et al.*, 1989a), however their effects may be complicated by possible dim light effects.

Form-deprivation myopia is axial in nature (Wallman *et al.*, 1978b; Troilo *et al.*, 1987; Schaeffel and Howland, 1988), with dramatic axial lengthening and marked corneal bulging being reported, although the corneal effect is inconsistent (Yinon *et al.*, 1982/1983; Hayes *et al.*, 1986). The magnitude of the deprivation response depends on the timing and duration of deprivation. Yinon (1980) found that form-deprivation myopia only occurred if the lid fusion was performed early in life (Yinon *et al.*, 1980). The presence of a short critical period for this response in the chick can be explained by the fact that the eyes of chicks, unlike mammals, have a relatively rigid ossicular system which may limit eye growth later in development. It has also been shown that deprivation-induced myopia is highly sensitive to periods of normal vision; even ten hours of deprivation each day causes little myopia when interrupted by brief periods of normal vision (Nickla *et al.*, 1989). Similarly, recovery from form-deprivation myopia has been shown to occur when normal vision is restored (Wallman *et al.*, 1981). During recovery, the growth rate of the vitreous chamber is subnormal until the eye recovers its normal proportions and refraction (Wallman and Adams, 1987).

Dark rearing

The effect of dark rearing on eye growth in chickens reinforces the importance of vision for normal ocular development. Dark rearing has been shown to cause increases in both axial and equatorial ocular dimensions, although the equatorial changes appear to dominate (Gottlieb *et al.*, 1985; Yinon and Koslowe, 1986; Osol *et al.*, 1986). Although the eyes are enlarged myopia does not develop due to the anterior chamber shallowing and corneal flattening; refractive errors are generally highly hyperopic (Gottlieb *et al.*, 1985; Yinon and Koslowe, 1986). Restoration of vision in chicks made hyperopic by dark rearing results in emmetropia due to vitreous chamber elongation (Troilo and Wallman, 1991). Normal vision in this case modulates eye growth in the opposite direction to that reported for recovery from form-deprivation, indicating that opposite growth signals are generated in response to the different types of refractive errors.

In chicks raised in darkness there is no significant difference between occluded and non-occluded eyes (Gottlieb *et al.*, 1987). Dark-reared chicks fail to develop myopia and can even develop hyperopia in response to lid suture (Yinon and Koslowe, 1986); this lends support to the proposal that the form-deprivation effect is primarily a visual one. However, an alternative interpretation is that the enlargement produced by dark rearing obscures the effects of deprivation. The effect of dark rearing on eye growth demonstrates that for the chicken, normal growth and form-deprivation effects are light-dependent.

Dim light

The intensity of light also affects the emmetropization process. Rearing under dim light conditions causes eye enlargement (Bercovitz *et al.*, 1972; Chiu *et al.*, 1975) both equatorially and axially, in both diurnal and constant dim light. The dim light effect is greatest when lights of restricted spectral ranges are used (Harrison *et al.*, 1968; Bercovitz *et al.*, 1972). Myopia and eye enlargement were consistently observed with the dim blue light condition used by Harrison *et al.* (1968) and Bercovitz *et al.* (1972) and small hyperopic refractive errors in dim white light reared chicks. Despite the similarities between dim and dark rearing effects, corneal flattening seen in dark reared chicks has not been consistently observed in chicks reared under dim light (Harrison *et al.*, 1968; Bercovitz *et al.*, 1972; Lauber and Kinnear, 1979). As the intensity of light is decreased the effects of lid suture on both axial and equatorial dimensions similarly decrease (Lauber and Oishi, 1987).

Continuous light

A third example of abnormal lighting conditions which affect eye growth, is rearing under continuous light. Paradoxically, like dim light and dark rearing, chicks raised under continuous light also develop greatly enlarged eyes; flat corneas, shallow anterior chambers, and elevated intraocular pressure are also observed. Eventually, continuous light rearing leads to blindness (Lauber, 1987). Taken together, form-deprivation, dark rearing, dim light and continuous light studies support high quality vision and appropriate lighting conditions as requirements for emmetropization.

Induced refractive defocus

The most conclusive evidence for a visual feedback system in chicks comes from spectacle lens studies (Schaeffel *et al.*, 1988; Irving *et al.*, 1991). Eye growth in the chick can be manipulated in either direction by spectacle defocus; functional hyperopia, produced by negative spectacle lenses,

induces myopic refractive errors while functional myopia, produced by positive lenses, induces hyperopia. Schaeffel *et al.*(1988) reported that the observed shifts in refractive state were always in the direction which compensated for the defocus provided by the lenses; refractive shifts of 38% to 51% of the inducing lens power resulted, with eyes wearing positive lenses being shorter than those wearing negative lenses. The authors suggested that 100% adaptation was prevented due to reduced retinal image quality with the lenses.

To investigate this phenomenon further, Irving *et al.* (1991) studied the effect of convex and concave lenses inserted into goggle mounts designed for chickens. After 2 weeks the eyes had developed refractive states equivalent in sign and magnitude to the lenses worn. Compared with Schaeffel *et al.*'s (1988) findings, they produced greater refractive changes and suggested that this was due to the application of the lenses at an earlier age, i.e. day 1 as opposed to 9 days post hatching. Irving *et al.* (1992) also experimented with soft contact lenses in chick and observed hyperopia during the first week presumably due to corneal flattening. Although refractive compensation was then good, increased axial dimensions were observed for both positive and negative lenses.

Due to the independent control of accommodative functions of the two eyes of chicks (Reiner *et al.*, 1983; Schaeffel *et al.*, 1986), it was suggested that the chick could keep both eyes in focus for the two different powered lenses and it was thus proposed that some of the compensation may have been due to differences in accommodative demand produced by the lenses (Schaeffel *et al.*, 1988). Accommodation effects have since been shown to be of little consequence, as compensation is still observed following lesions of the Edinger-Westhphal nucleus (Schaeffel *et al.*, 1990) and section of the optic nerve (Wildsoet and Wallman, 1992).

These results indicate that in the chick there may two growth signals: one to increase growth and the other to decrease it. The signal which is activated during lens wear governs the resultant eye growth observed.

1.2.2. Local Control of Eye Growth

Emmetropization in chicks is thought to be primarily under local ocular control. Evidence for local control of ocular growth includes: asymmetric eye growth in response to sectional occluders (Wallman and Adams, 1987) and low-ceilings (Miles and Wallman, 1990), optic nerve section (Wildsoet and Pettigrew, 1987; Troilo and Wallman, 1991), ciliary nerve section (Wildsoet and Howland, 1991) and Edinger-Westphal ablation (Troilo and Wallman, 1988). Myopia in chicks raised with white translucent occluders

covering only part of the eye was restricted to the visually deprived part of the retina; the non-deprived part remained nearly emmetropic (Wallman and Adams, 1987). Correspondingly, the vitreous chamber depth enlarged only in the deprived region, the net effect being asymmetric eye shapes. When the occluder was removed, a rapid return to ocular symmetry and a normalization of refraction in the previously myopic region of the eye occurred (Wallman and Adams, 1987). The recovery process observed after full occlusion also occurs after partial deprivation, but is confined to the previously deprived segment. Miles and Wallman (1990) showed that chicks raised in low-ceiling environments become more myopic in the upper visual field than chicks raised in high-ceiling environments, the sectional change in refraction resulting from a selective enlargement of the vitreous chamber in the ventral region (Miles and Wallman, 1990). These results imply that local regions of the retina can control the growth of the subjacent sclera. Also, a local ocular phenomenon, i.e. the movement of the retina by changes in choroidal thickness, has been suggested as a means of focussing the eye (Wallman *et al.*, 1992).

Form-deprivation myopia and recovery from deprivation were not prevented by eliminating accommodation by lesioning of the Edinger-Westphal nucleus (Troilo and Wallman, 1988), nor by sectioning of the ciliary (Wildsoet and Howland, 1991) or optic nerves (Wildsoet and Pettigrew, 1987; Troilo and Wallman, 1991). This suggests that there are eye growth mechanisms that are not dependent on central control. However, optic-nerve-sectioned eyes tend to overshoot emmetropia (Wildsoet and Pettigrew, 1988a; Troilo and Wallman, 1991). This implies that although the isolated eye can sense the sign of defocus, the brain may play some role in determining the magnitude of response.

As eye growth is thought to be locally mediated this leads to the question of which retinal neurons are involved in regulating growth. It seems unlikely that ganglion cells have a key role, as form-deprivation myopia still develops following the elimination of ganglion cells by optic-nerve section (Troilo *et al.*, 1987; Wildsoet and Pettigrew, 1987). It has been suggested that amacrine cells are the most likely candidates (reviewed in Wallman, 1991). Neurotoxin studies support this hypothesis; kainic acid injections produce vitreous chamber enlargement (Wildsoet and Pettigrew, 1988b; Barrington *et al.*, 1989) while injections of quisqualic acid decrease vitreal growth and deepen the anterior chamber (Barrington *et al.*, 1989). Retinal assays have shown that visual deprivation decreases retinal dopamine levels in the chick; consistent with this, apomorphine injections decrease the effects of deprivation (Stone *et al.*, 1989).

1.3. Myopia in other Animals

Myopia has also been induced in a variety of other animal species (for reviews see Curtin, 1985; Goss and Criswell, 1981; Criswell and Goss, 1983; Yinon, 1984 and Smith, 1991). Monkeys, tree shrews and cats all become myopic in response to visual deprivation. Retinal image degradation, achieved by either eyelid suture, corneal opacification or translucent occluders, is a common experimental manipulation used to induce myopia in animals; interference with form vision disrupts emmetropization.

1.3.1. Monkey

The idea of a visual feedback system for eye growth is supported by primate studies. Greene and Guyton, (1986) observed an exponential decrease in the normal refractive error of monkeys, while deprivation induced axial myopia (Wiesel and Raviola, 1977; reviewed in Raviola and Wiesel, 1990), the magnitude of which was related to the age of the monkey and to the duration of deprivation (Smith *et al.*, 1987). Initial studies used lid suture; however responses were unreliable and more variable compared with responses of chick. Deprivation effects were also observed in monkeys with corneas made opaque by the injection of small polystyrene beads (Wiesel and Raviola, 1979), indicating that the axial myopia was not an artifact of the surgery, e.g. due to temperature elevation under the closed lid (Raviola and Wiesel, 1985). Retinal light stimulation through the closed lid was a requirement for myopia development. Similar to chicks, monkeys reared in the dark failed to develop lid suture myopia and in many cases hyperopia developed (Raviola and Wiesel, 1978). Dark rearing also interfered with the emmetropization process in the rhesus monkey; this observation represents further evidence for emmetropization being vision dependent in primates (Guyton *et al.*, 1989).

In primates myopia caused by deprivation persists following restoration of normal vision, even if this occurs before adult eye dimensions are reached; there is no attempt to compensate for the excessive length of the vitreous chamber caused by deprivation (Wiesel and Raviola, 1977). This is in contrast to the chick where refractive errors

decrease when vision is restored (Wallman *et al.*, 1981) and may indicate that in primates, visual factors can only act to increase growth rates.

Whether accommodation is a factor in refractive error development in the monkey is an unresolved issue. Conflicting results have been reported for different monkey species, e.g. lid-sutured arctoide monkeys do not develop myopia when the ciliary nerve is paralysed or the optic nerve cut, whereas in rhesus monkeys, these procedures do not prevent form-deprivation myopia (Raviola and Wiesel, 1985; reviewed in Raviola and Wiesel, 1990). Similarly atropine is effective in preventing lid-suture myopia in stumptail macaques but not rhesus macaques (Raviola and Wiesel, 1985). Presented as evidence for a relationship between myopia and near-visual tasks is the substantial increase in myopia reported in monkeys reared in a near-visual environment (Young, 1963). However, the environment used was white and unpatterned and thus the myopia may have resulted from a type of form deprivation rather than hyperopic defocus and excessive accommodation.

In contrast to chicks, evidence for bi-directional control of refractive errors, using positive and negative spectacle lenses, has yet to be demonstrated in primates. Studies on monkeys involving lens-induced defocus are difficult to interpret. Monkeys reared wearing contact lenses tend to develop a relative hyperopia in their treated eye regardless of the sign of defocus (Crewther *et al.*, 1988). This may be due to the young age at which lens wear was commenced, as it has been reported that humans who develop visual impairment before three years of age develop hyperopia (Nathan *et al.*, 1985).

1.3.2. Tree Shrew

An advantage of the tree shrew as a model is its rapid maturation time (3 to 6 months), classification as a "primitive primate" and reliable response to lid suture (Sherman *et al.*, 1977). Significant axial myopia (average ≈ –20 D) occurs in response to form deprivation, with the myopia largely being attributed to observed vitreous chamber elongation (Norton, 1990). Partial neutralization of vitreous effects by corneal flattening (Norton and McBrien, 1992; Marsh-Tootle and Norton, 1989) and lens and zonular hypoplasia have also been described (McKanna and Casagrande, 1978). McKanna and Casagrande (1978) interpreted zonular and lens hypoplasia as proof of sustained accommodation during lid suture and support for their proposal that lid suture simulated empty field conditions and

stimulated accommodation (Whiteside, 1952; Westheimer, 1957). Accommodation was suggested to underlie the myopia observed in the tree shrew. Consistent with this interpretation, chronic cycloplegia blocks form-deprivation myopia in this mammal (McKanna, 1982; McKanna and Casagrande, 1985). However, paradoxically, dark rearing, which is likely to have effects on accommodation similar to those of the empty field condition (Leibowitz and Owens, 1975; Leibowitz and Owens, 1978) is also effective in blocking form-deprivation effects (McKanna, 1982; McKanna *et al.*, 1983).

Form-deprivation myopia can be reversed in the tree shrew but only if normal vision is restored during a narrow age window (Norton, 1990). While eye growth appropriate to the sign of defocus appears to occur in response to negative spectacle lenses, both hyperopic and myopic defocus produce vitreous chamber elongation and myopia (Siegwart and Norton, 1993). The results show the importance of vision for normal eye growth but do not conclusively establish a role for accommodation in form-deprivation myopia in tree shrews.

1.3.3. Cat

In comparison to monkeys and tree shrews, the magnitude of deprivation-induced refractive errors in kittens are small and there are differences in the type of refractive errors produced (Kirby *et al.*, 1982; Yinon, 1984; Gollender *et al.*, 1979; Smith, 1981; reviewed by Smith, 1991). Yinon (1984) has attributed these results to differences in the lids of cats compared with other animals; the limited capacity of light to penetrate the closed lid of the cat (no greater than 5% transmission, (Crawford and Marc, 1976)) has been well documented (Loop and Sherman, 1977) and could mean that in many instances very dim or no light reached the retina. Thus lid suture simulated dark rearing.

Some authors (reviewed in Goss and Criswell, 1981), have attributed the lack of response to lid suture to the cat's limited accommodative capacity. Other studies have sought to link myopia and accommodation; increased accommodative activity in cage-reared cats has been suggested as the reason for the refractive differences between "street" and cage-reared animals (Rose *et al.* 1974; Yinon 1984). Similar to the studies of near environment rearing in monkey there are other interpretations of these results.

The results of refractive defocus experiments in cats are more difficult to interpret than those for chicks. Smith *et al.*, (1980) performed

the first investigation on the effects of optical defocus. Kittens were reared with a monocular high powered minus spectacle lens (–10 D to –16 D) which, due to consensual accommodation, was thought to produce a defocussed image in the lens wearing eye. Kittens wore the lens for 2 to 3 hours per day and were kept in total darkness the rest of the time; a myopic shift in the treated eye was exhibited after 8 weeks of wear. However, the myopia may have occurred due to the significant period of dark rearing rather than as a consequence of the applied defocus; eye enlargement has been reported in dark-reared chicks. In a more recent follow up study (Ni and Smith, 1989), it was determined that when the degree of defocus was large (≥ 10 D) the defocussed eye developed an axial myopia. Small amounts of optical defocus (3 D to 4 D) failed to produce consistent changes. In contrast, no systematic alterations in refractive error were observed in kittens reared with hard contact lenses rather than spectacle lenses (Nathan *et al.*, 1984) and it was concluded that myopia could not be reliably induced in kittens by optical defocus. Ni and Smith (1989) suggested that subthreshold defocus levels were the explanation for the negative results obtained by Nathan *et al.* (1984). The results suggest that large amounts of optical defocus are required for the disruption of emmetropization in kittens. Visual regulation of eye growth in the cat is suggested by studies showing axial elongation in response to reductions in optical power produced by radial keratotomy (Hendrickson and Rosenblum, 1985).

1.4. Possible Visual and Behavioural Cues for the Control of Eye Growth

Many different theories have been postulated for how the eye senses its refractive error and directs its growth towards emmetropia. Both "passive" and "active" models for eye growth control have been proposed for the chick. In the passive model the axial growth of the eye is linked to the reduction in hyperopia seen at hatching and, in addition, compensates for the progressive corneal flattening that occurs with growth. Presumably, when emmetropia is reached, the increased sharpness of the retinal image and increased retinal activity generate a yet to be identified signal to "stop growth". Proportional "passive" eye growth cannot account completely for the emmetropizing growth in the young chick (Wallman and Adams, 1987). The second "active" model involves the eye responding to a signal proportional to the magnitude and direction of the refractive error (Schaeffel and Howland, 1991; reviewed by Wallman, 1991). There is increasing evidence for the latter

mechanism in the chick. The different time courses of "developmental emmetropization" and emmetropization in response to induced defocus is a puzzle; "developmental emmetropization" usually occurs over many weeks while lens adaptation occurs within a few days. As a possible explanation, it may be that the usually small, naturally occuring refractive errors in chicks are not great enough to initiate the active, faster emmetropization mechanism.

While the studies described imply that emmetropization is visually guided and that the eye can discern the sign of defocus, the nature of the visual cues to defocus are unknown. Any eye growth mechanism must be detected locally within the eye because i) form-deprivation myopia develops (Wildsoet and Pettigrew, 1988a) and the correct response for spectacle defocus occurs (Wildsoet and Wallman, 1992) when the eye is disconnected from the brain by optic-nerve section, and ii) myopia can be induced in local retinal regions in response to segmentary deprivation (Wallman *et al.*, 1987). The visual cue must be able to be processed locally within the retina and the retina must be sensitive to both the magnitude of blur and the sign of defocus (Schaeffel *et al.*, 1988).

Some possible visual or behavioural cues to defocus include: retinal activity, accommodation, chromatic aberration, contrast, spatial frequency, astigmatism and spherical aberration. Evidence both for and against each of these being involved in emmetropization is discussed. While each factor is discussed separately, it is of course possible that a number of cues are used simultaneously to guide emmetropization.

1.4.1. Retinal Activity

Is the growth of the eye controlled by the overall retinal neural activity? A hypothesis pursued in work in this thesis is the proposal that retinal neural activity is maximal when an image is sharply focussed on the retina, as contrast is maximal and neurons responding to high spatial frequencies are active. This increased neural activity might be the "stop" signal for eye growth. In the open loop conditions of visual deprivation, this "stop" signal would be absent, causing growth to proceed unchecked. Results of stroboscopic light studies are consistent with the hypothesis that increased retinal activity is associated with decreased scleral growth (Wallman, 1991). Chicks occluded under stroboscopic light are much less myopic than those deprived under normal light conditions (Gottlieb and Wallman, 1987). It has been suggested that this is due to increasing the number of temporal retinal transients, i.e. temporal activity (Gottlieb and

Wallman, 1987). It has been shown that stroboscopic illumination is most effective at reducing form-deprivation myopia between 10 and 20 Hz (Vingrys *et al.*, 1991), which is compatible with it acting via a neuronal mechanism. However, this interpretation is perhaps too simplistic as enormous variations in refractive error are observed with stroboscopic lighting conditions. Overall retinal activity cannot be the only factor involved in eye growth control.

The retinal activity theory could also account for the association of large amounts of reading with myopia. Wallman *et al.* (1987) pointed out that as there is relatively very little low spatial frequency information in text, only the central retinal neurons could respond to the high frequency information presented. Thus, during reading, only these central neurons would be highly active. Other neurons would experience little stimulation, overall retinal activity would be low and erroneous defocus signals could be activated.

1.4.2. Accommodation

When an observer shifts his gaze from a distant to a near object, the refractive power of the eye increases so as to maintain a focussed retinal image; this refractive change is known as accommodation. There are two main issues in any discussion involving the role of accommodation in emmetropization: does the "accommodation signal" feed into the emmetropization system, and are the microfluctuations of accommodation involved in sensing defocus?

The average level of accommodation is a function of the eyes' refractive state; hyperopic eyes accommodate more than myopic eyes. It has been suggested that the average magnitude of accommodation may modulate eye growth and that this may also explain the refractive compensation for spectacle lenses (Schaeffel *et al.*, 1988). If axial elongation depended on the magnitude of accommodation then hyperopic eyes would elongate more quickly than myopic eyes, and in both cases ametropia would be reduced. This system would presumably also require a "summation system" to determine the average level of accommodation per day, with accommodation above some criterion amount causing accelerated eye growth. It has been suggested that accommodation driven anatomical changes could mechanically increase eye growth (Coleman, 1970).

This accommodation model could be used to explain the link between "excessive" nearwork and myopia. While emmetropia is the

expected endpoint of this process, excessive use of accommodation under other circumstances, e.g. with prolonged near activities, could result in an erroneous hyperopic defocus signal and myopia production. The greater than normal "accommodation signal" driving eye growth in the direction to decrease the need for high levels of accommodation. Unfortunately, this model fails to explain the inadequacy of myopia treatments designed to slow myopia progression by reducing accommodation.

Currently, there are no models available to describe how the accommodative tonus might influence eye growth of chickens. Schaeffel *et al.* suggested a mechanism that includes changes in intraocular pressure, resulting from changes in accommodative tonus (Schaeffel *et al.*, 1988; Schaeffel and Howland, 1991). Experiments in chicks, which show that emmetropization still occurs in response to spectacle defocus following lesions of the Edinger-Westphal nucleus (Schaeffel *et al.*, 1990) and following ciliary nerve section (Wildsoet and Howland, 1991) also imply that accommodation is not essential for emmetropization.

Many studies have shown that changes in retinal image quality are among the most important factors in accommodation control (Smithline, 1974). It has been suggested that accommodative fluctuations might be used as an error detector for the control of accommodation (Alpern, 1958; Fender, 1964). Accommodative fluctuations could be used to extract directional information from a nondirectional blur signal (Fincham, 1951; Smithline, 1974) and may be involved in sensing defocus.

While accommodation may not be essential for emmetropization, it must be taken into account during normal ocular development as its action ensures a focussed retinal image under normal conditions. As the defocus cue that guides accommodation and that used by the emmetropization system may be similar, some of the discussions that follow identify likely cues for emmetropization in relation to the accommodation system.

1.4.3. Longitudinal Chromatic Aberration

Longitudinal chromatic aberration is due to variations in refractive index with wavelength and is observed only in polychromatic light (Fig. 1.4). The human eye possesses approximately 2 D of longitudinal chromatic aberration across the visible spectrum (Wald and Griffin, 1947; reviewed by Charman, 1983). Chromatic aberration is the dominant source of optical blur for pupil sizes greater than 4 mm (Campbell *et al.*, 1990).

In relation to accommodation control, Fincham (1951) suggested that longitudinal chromatic aberration signalled the direction of defocus, on the basis that 60% of his subjects failed to respond or only partially responded to an accommodative target presented under monochromatic light (Fincham, 1951). This is supported by other studies showing that some humans show poor or absent dynamic accommodation responses under monochromatic conditions (Kruger and Pola, 1986; Kruger *et al.*, 1993), as do monkeys (Flitcroft and Judge, 1988). The recent findings of Switkes, *et al.* (1990) showed that colour contrast is an ineffective accommodative stimulus for the human accommodation system.

Similarly for emmetropization, the difference in focus of different wavelengths of light could provide a defocus cue; longer wavelengths (red) will be clearer than shorter wavelengths (blue) for myopic eyes and for hyperopic eyes the reverse will occur. Fincham (1951) also observed coloured fringes around targets viewed under white light; these changed with focus such that a blue band appeared on the outside of the fringe for the myopic state and a red band for the hyperopic state.

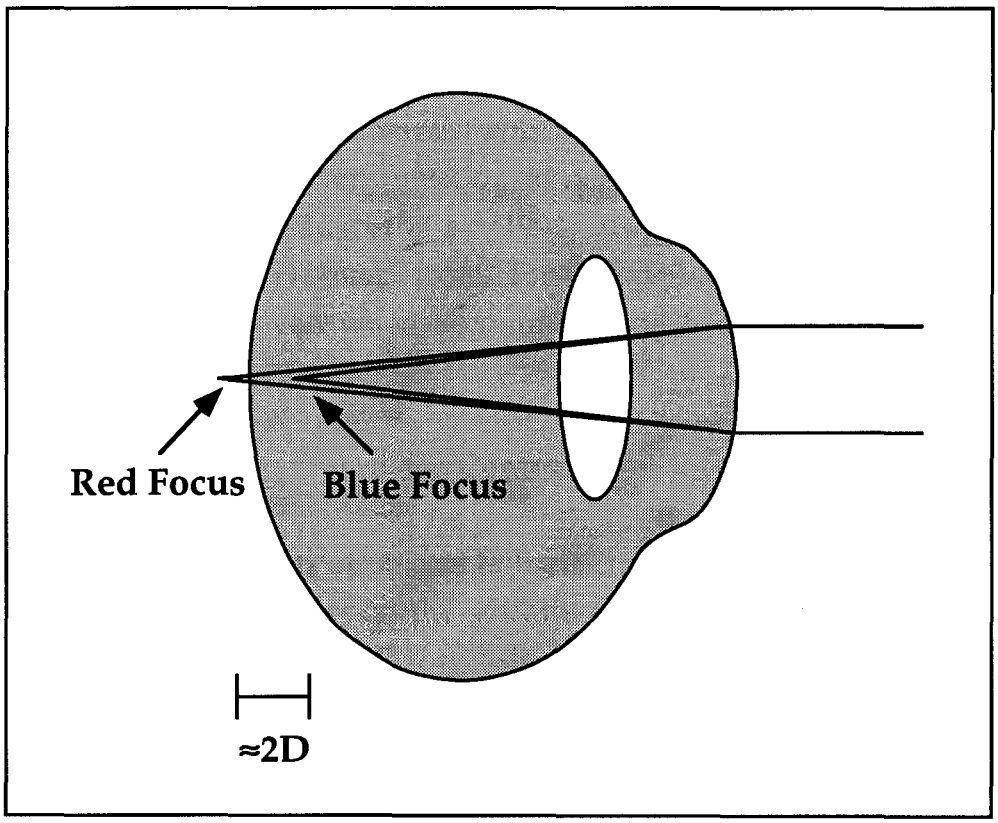


Figure 1.4. Schematic representation of the chromatic aberration of the relaxed human eye. The human eye typically possesses 2 to 3 D of chromatic aberration.

The cue for emmetropization must be detectable by the visual system at an early age. Colour vision seems to develop early in infants with behavioural studies reporting the emergence of colour vision in infants between 1 and 3 months of age (Teller *et al.*, 1978; Hamer, *et al.*, 1982; Varner *et al.*, 1985; reviewed in Brown, 1990 and Morrone *et al.*, 1993), although this is later than the development of luminance contrast sensitivity (Allen *et al.*, 1993). It has been recently reported that human infants have functional medium-wavelength-sensitive and long-wavelength-sensitive cones and the required post-receptor chromatic mechanisms to compare their signals (Allen *et al.*, 1993).

It seems reasonable to suggest that the emmetropization system, like the accommodation system, may use chromatic aberration as a cue to defocus and that a similar mechanism may be used in chicks. It has been reported that the chick eye possesses approximately 1.25 D of longitudinal chromatic aberration (Sivak and Mandelman, 1982). To examine the role of longitudinal chromatic aberration in determining the sign of refractive defocus, Schaeffel and Howland raised chicks with + 4 D and – 4 D lenses in monochromatic light (low pressure sodium lamps, 589 nm). They found no difference from results obtained under white light with refractive compensation, i.e. hyperopia, in response to positive lenses and myopia for negative lenses. It would appear that in chicks, chromatic cues are not necessary for emmetropization to occur (Schaeffel and Howland, 1991). Recent data of Wildsoet *et al.* (1993) also support this view. They showed that chicks recover from form-deprivation myopia under monochromatic light even when accommodation has been eliminated as an alternative cue.

1.4.4. Contrast

Crucial for the perception of objects is their contrast, i.e. the difference in the intensity of adjacent areas; the visibility of objects depends on their contrast with their background. Increasing levels of defocus are associated with increasing levels of blur and a loss of contrast. Could low contrast serve as a signal for defocus? If low contrast were a signal for increased eye growth this could explain the myopia seen with form deprivation.

In relation to accommodation control, several studies have examined the effects on accommodation of presenting low contrast targets. Heath (1956) demonstrated that spatial contrast was an important accommodative stimulus (Heath, 1956); a reduction in image contrast

resulted in a flattening of the stimulus/response curve (Wolfe and Owens, 1981). However, other results indicate that the accommodation response is not greatly influenced by contrast (Charman and Tucker, 1978b; Ciuffreda and Rumpf, 1985; Tucker *et al.*, 1986). The finding that humans can accurately accommodate to a sinusoidal grating (Charman and Tucker, 1977; Owens 1980), where defocus attenuates contrast but not the shape of the waveform, has raised the possibility that accommodation operates as a contrast maximizing feedback loop (Kotulak and Schor, 1986b). Kotulak and Schor (1986b) suggested that an increase in retinal-image contrast associated with increased accommodation signalled that an increase in lens power was required to improve focus and a decrease in contrast with increased accommodation signalled the reverse.

In addition, sinusoidal oscillations of accommodation, i.e. microfluctuations, at a fixed amplitude produce sinusoidal variations in contrast, the amplitude and modulation of which is determined by the magnitude of defocus (Fincham, 1951; Smithline, 1974). Perhaps the sinusoidal temporal variations in contrast associated with microfluctuations could be used to guide emmetropization.

In light of evidence that local retinal mechanisms underlie emmetropization, the visual cue for emmetropization must be detectable by the retina. As a visual cue, changes in contrast meets this criterion. Between the photoreceptors, which are involved in the initial detection phase, and the ganglion cells, which transmit the visual signal to the brain, complex visual processing occurs. The retina processes contrast information which is degraded by defocus and thus the retina has access to defocus information. It is well established that ganglion cells are able to relay information about the shape, colour and contrast of the visual image (DeMonasterio and Gouras, 1975; Shapley and Perry, 1986; reviewed in Shapley and Enroth-Cugell, 1984). Of relevance to the current discussion, two broad classes of ganglion cells, one with high luminance-contrast sensitivity and the other with low luminance-contrast sensitivity have been described in monkey retina (Kaplan and Shapley, 1986).

1.4.5. Spatial Frequency

What spatial frequencies are important for visual regulation of eye growth? The conventional view is that the eye changes focus or alters growth, so that fine-detailed images remain clear on the retina. In this model, fine details, i.e. high spatial frequency components of the image

would be important. Alternately, as recognition of large objects is much less impaired than that of small details by errors of focus, low spatial frequencies could be used initially to guide growth when large amounts of blur are present and high spatial frequencies could be used to "fine tune" the response.

While the spatial-frequency dependence of the eye-growth control system is unknown, information is available about the accommodation system. Early studies supported the view that high spatial frequencies were required for accurate accommodative responses (Charman and Tucker, 1977). However, later studies suggest that optimal accommodative performance requires intermediate frequencies between 3 and 5 cycles/degree (Owens, 1980; Bour, 1981). Emmetropization, like accommodation, may be tuned to intermediate spatial frequencies, where the visual system is most sensitive to changes in image contrast (Ciuffreda and Hokoda, 1983).

Of possible relevance here is the observation by Ni and Smith (1989), that a large degree of optical defocus is required to disrupt the emmetropization process of kittens. It could be argued that, as defocus acts as a high spatial frequency filter, the emmetropization mechanisms in cats do not require high spatial frequency information and/or high image contrast.

Recognition of large objects is much less impaired than that of small details by errors of focus. Likewise, occluders have been demonstrated to reduce both contrast and high frequency information. However, while it has been suggested that the axial elongation and myopia produced by occluders is due to image degradation (Hodos and Kuenzel, 1984), it is not clear whether the loss of contrast or the loss of spatial frequencies is more important.

1.4.6. Astigmatism

Astigmatism usually results from the toricity of one or more of the eye's refracting surfaces. A spherical optical system forms a point image of a point object but in regular astigmatism, the image of a point consists of two line foci at different distances and at right angles to each other. The meridians containing the two line foci are known as the principal meridians of the eye and the separation of these foci is called the interval of Sturm (Fig. 1.5).

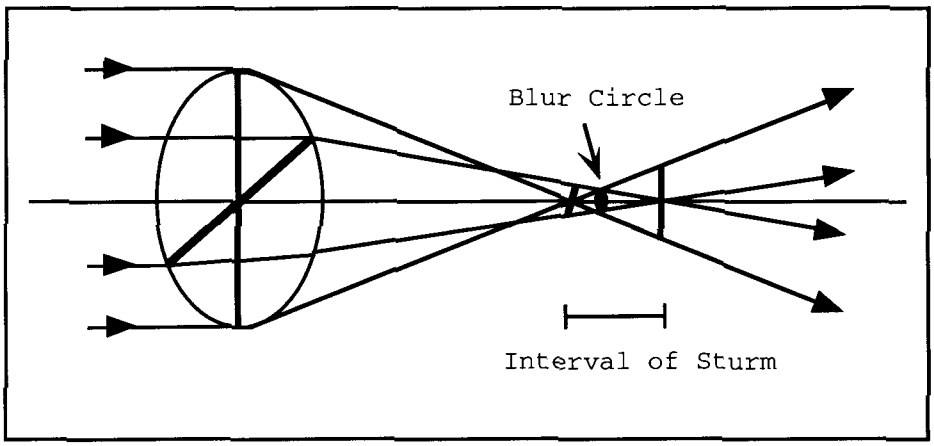


Figure 1.5. Ray diagram depicting the two line foci produced by a toroidal surface.

Astigmatism has been suggested as a possible cue for the accommodation system. Campbell (1958) reported that subjects were unable to detect the direction of defocus unless astigmatism was present, astigmatism acting as a cue to accommodative tracking in some subjects (Campbell and Westheimer, 1959). Over 70% of the adult population have astigmatism of greater than 0.25 D (Saunders, 1981). The refractive separation of the line foci may provide information about direction of both accommodative defocus and required eye growth, i.e. one of the line foci may appear clearer than the other and provide the directional cue.

Human studies indicate a higher incidence of high astigmatism in infants under 12 months of age when compared with adults. While the magnitude of astigmatism rapidly decreases during the second year of life (Howland *et al.*, 1978; Atkinson *et al.*, 1980; Fulton *et al.*, 1980; reviewed in Banks, 1980; Mohindra *et al.*, 1978), uncorrected astigmatism in childhood has been proposed as a cause of myopia (van Alphen, 1961; Fulton *et al.*, 1982; reviewed by Lyle, 1991). Birnbaum (1978) specifically linked against-the-rule astigmatism with the development of myopia. Myopia might occur in the case of uncorrected astigmatism from the excessive and fluctuating accommodation required to bring each of the line foci in turn into focus.

It has been suggested that astigmatism could provide defocus information for the eye growth mechanism (Wallman, 1993). For low levels of defocus which shift the image plane either side of the best plane of focus, depending on the type of astigmatism, different meridians would have different clarity. For against-the-rule astigmatism, myopic defocus

would result in the image of a round spot appearing horizontally oval and for hyperopic defocus it would appear vertically oval. Orientation-sensitive neurons may then be able to discern the sign of defocus however the evidence for the existence of such cells is sparse (Maturana and Frenk, 1963).

1.4.7. Spherical Aberration

Longitudinal spherical aberration is defined as the axial separation of the paraxial and marginal focal points. The spherical aberration is positive when the focal distance of marginal rays is shorter than that of paraxial rays.

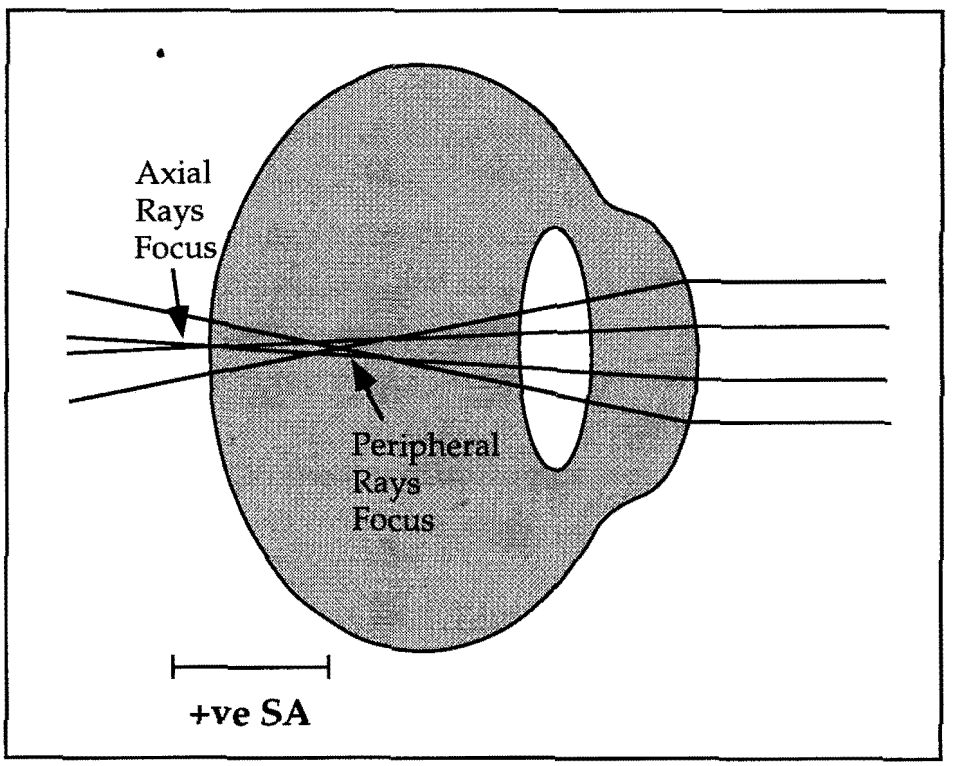


Figure 1.6. Schematic representation, showing the formation of positive spherical aberration (SA) most commonly seen in the relaxed human eye. Peripheral light rays come to a focus before the axial rays.

In humans, the unaccommodated eye tends to exhibit positive spherical aberration (Fig. 1.6). However, during accommodation spherical aberration becomes less positive or even negative. Spherical aberration is

considered to be the dominant monochromatic on-axis aberration. The magnitude of this aberration varies widely among humans (Koomen *et al.*, 1949; Ivanoff, 1956) and is lower than that predicted by schematic eye models largely due to the asphericity of the optical surfaces and to the index gradient in the lens (reviewed by Charman, 1983). Campbell and Westheimer (1959) suggested that spherical aberration could be used as a cue to accommodative tracking in some subjects. Perhaps the eye could use the difference in focus of axial and peripheral light rays as a focus cue for the guidance of eye growth. Analysis of the photoretinoscopic reflex and schematic eye modelling has demonstrated that the 30 day old chick eye possesses 0.5 D of spherical aberration (Schaeffel and Howland, 1988a) which could be used for this purpose.

1.4.8. Perceptual Factors

In humans, perceptual cues such as changes in image size, brightness, interposition and perceived distance provide information about distance to a viewed object. Changes in accommodation with dynamic changes in size, i.e. object looming, have been observed (Kruger and Pola, 1985; McLin *et al.*, 1988) and suggest that at least some of these cues are used by an ocular control system. As the distance to a viewed object decreases, blur increases for hyperopes and decreases for myopes. Perhaps, by using one of the above perceptual cues, the direction of change in object distance can be determined and by comparison with changes in retinal blur, appropriate growth responses made.

1.4.9. Foveal Pit

It is thought that the chameleon uses its deep foveal pit to convert blur into a movement cue, the direction of which gives the sign of defocus (Harkness, 1977; Harkness and Bennet-Clark, 1978). This is an unlikely mechanism for the chick, given its afoveate area centralis (Morris, 1982) and the ability of local regions of the retina to control the growth of the subjacent sclera (Wallman *et al.*, 1987).

1.5. Issues Addressed

On the basis of the research reviewed, it seems plausible that, at least in the chick, the eye is able to determine its refractive error and make the appropriate eye growth response to eliminate this error. The mystery is what defocus signals are used in this emmetropization process. In Chapter 2, the effect on form-deprivation myopia and emmetropization of the physiological variants, breed, sex and eye treated are investigated. In Chapter 3 the sensitivity of the chick eye to different visual disturbances is studied, with particular reference to the effect of normal vision on form-deprivation myopia and compensation to spectacle lenses and the role of depth-of-focus in emmetropization. The remainder of the thesis investigates some of the cues that could be used to control eye growth, in particular accommodation, chromatic aberration, contrast, spatial frequency and astigmatism. The relationship of the results to the theory that nearwork myopia is caused by an erroneous defocus signal rather than excessive accommodation is also discussed. In the final chapter a preliminary model for the refractive error detector guiding emmetropization in the chick is proposed. The motivation for this study is that only when the mechanism of myopia development is understood will it be possible to prevent or to reduce the occurrence of refractive anomalies rather than simply neutralize them with optical devices.

CHAPTER 2

EFFECT OF PHYSIOLOGICAL VARIANTS ON EYE GROWTH AND FORM-DEPRIVATION MYOPIA

2.0. Effect of Physiological Variants on Eye Growth and Myopia

Lid-suture myopia in chicks was first reported by Wallman *et al.* (1978b) who suggested that a common mechanism was involved in both lid suture and occlusion effects. This provided the impetus for many more studies investigating eye growth in chicks. Many different breeds of chicks have been used and while some studies report which eyes were treated and the sex of chicks used others do not.

This chapter deals with investigations critical for the correct experimental design of experiments involving the chick as a model for ocular growth. In section 2.1 the significance of the breed of chick used for eye growth studies was investigated, in section 2.2 the significance of the sex of chick studied, i.e. cockerel compared with pullet, was investigated and in section 2.3, the significance of which eye was treated, i.e. right or left, was studied.

2.1. The Significance of Breed of Chick

2.1.0. Summary

The effects of form deprivation were compared in two breeds of chicks: i) White Leghorn and ii) broiler cross. While both breeds showed high myopia and axial elongation in response to either lid suture or occlusion, they differed significantly in the magnitude of this response. The speed and magnitude of recovery from myopia with restoration of normal vision was also breed-dependent.

2.1.1. Introduction

Over the last 20 to 30 years, there has been a great deal of interest in the development of refractive errors and in particular, the mechanisms underlying the development and progression of myopia. Both animal and human research has been used to study this complicated problem.

Monkeys (reviewed in Raviola and Wiesel, 1990) tree shrews (reviewed in Norton, 1990) and most commonly chicks (reviewed in Wallman, 1993) are used as animal models for myopia. Many different breeds of chicks, e.g. White Leghorn (Yinon *et al.*, 1980; Osol *et al.*, 1986; Wildsoet and Pettigrew, 1988) broiler (Lauber and Oishi, 1987), New Hampshire and White Rock-Rhode Island Red cross (Hayes *et al.*, 1986), have been used for eye-growth studies. While in most studies the breed of chick used is stated, in others it is not (Wallman *et al.*, 1978b; Pickett-Seltner *et al.*, 1988). Chicks are sometimes simply referred to as "domestic" chicks (Yinon *et al.*, 1982/1983; Lauber and Oishi, 1987). Also, results obtained using different breeds of chicks are often compared without reference to breed. The hypothesis that eye-growth patterns in chickens are breed dependent, and, in particular, that significant breed-related differences in overall body growth rates may be mirrored in differences in i) normal eye growth and ii) susceptibility to and recovery from form-deprivation myopia was investigated.

2.1.2. Methods

Animals and treatments

The eye-growth patterns of two breeds of chickens: i) White Leghorns (generally bred as egg layers) and ii) broilers ("meat birds") were studied. Both day-old Male White Leghorn (WL) and broiler (BR) chicks had one eye sutured closed (LS; WL, n=8; BR, n=6) or a translucent occluder (OC; WL, n=8; BR, n=6) glued over one eye for two weeks, i.e from day 1 to 14. The recovery process was then monitored for a further 4 weeks. Chicks were raised in temperature controlled enclosures with food and water provided *ad libitum*. They were exposed to a 12/12 light-dark cycle, with lights on at 7 am and off at 7 pm and light intensity of 250 lux at the level of the food trough.

Measurements

Eye growth was monitored weekly for 6 weeks. Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed under dim illumination to determine the refractive error and the positions of the intraocular surfaces respectively. Refractive error, anterior chamber depth (ACD), axial lens thickness

(ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained for both treated and normal eyes. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987), under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test was used (WSRT). To assess the differences between the two breeds of chicks the Mann-Whitney U-test was used (MWUT). To compare treatment effects, interocular differences were used. Dimensional changes between measurement points were used as estimates of growth. Eye volumes were calculated using AL data and approximating the eye to a sphere. All data reported in the results section are in the form mean ± SD unless otherwise stated.

2.1.3. Results

Normal ocular development

The refractions of normal eyes at the earliest measurement point, week 1, were significantly more hyperopic for the WL compared with BR chicks, i.e +3.1±0.4 D compared with +1.1±0.6 D ($P < 0.005$, MWUT; Fig. 2.1.1; see Appendix II, Tables AII.2.1, for treated and normal eye data). This difference did not persist with low hyperopic refractive errors being measured at later ages for both breeds. At the last measurement point, week 6, refractions were only very slightly hyperopic, i.e. +0.2±0.7 D and +0.9±1.1 D for WL and BR groups respectively. Although at week 1 the breeds differed in refractive error, there was no significant difference in their ALs at either weeks 1 or 2 (Fig. 2.1.1). However, from week 3 onwards the AL of BR chicks was significantly greater than that of WL chicks. At the last measurement point, week 6, ALs were 11.84±0.21 mm and 12.37±0.24 mm for WL and BR groups respectively; in percentage terms, BR eyes were 4.8% greater in length than WL eyes, at week 6.

The difference in measured AL between the breeds reflected differences in ACD and VCD growth (Fig. 2.1.2). From week 3 onwards, the ACDs of BR chicks were significantly deeper than those of WL chicks. The ACD at week 6 was 2.02±0.05 mm for WL chicks and 2.29±0.09 mm

for BR chicks, i.e. the ACD of BR chicks was 13.4% greater than that of WL chicks. Similarly, the vitreous chamber was significantly deeper in BR chicks at all measurement points, the difference between breeds increasing with increasing age, e.g. the VCD of BR chicks was 4.8% greater than that of WL chicks, 4.99±0.09 mm compared with 5.23±0.10 mm at week 1, and 7.8% greater, 6.83±0.17 mm compared with 7.36±0.19 mm at week 6.

In contrast and partly offsetting the effects on AL due to the disproportionate growth of the anterior and vitreous chambers, the lens was consistently thinner for BR chicks compared with WL chicks at all measurement points; the difference in percentage terms was consistent across all ages. At week 1, the ALT was 2.18±0.03 mm for WL chicks compared with 1.98±0.02 for BR chicks; WL lenses were 10.1% thicker than BR lenses. Similarly, at week 6 measurements were 2.98±0.04 mm and 2.71±0.03 mm respectively, with the lens of WL chicks being 10.0% greater in thickness than that of BR chicks.

The cornea of WL chicks was initially slightly flatter than that of BR chicks and then became slightly steeper over time (Fig. 2.1.3). At week 6 the cornea of WL chicks was significantly steeper (2.4% steeper) than that of BR chicks; corneal powers of 84.1±0.9 D were observed for WL chicks compared with 82.1±1.1 D for BR chicks ($P < 0.05$, MWUT). In summary, BR and WL chicks were both emmetropic from week 2; BR chicks had significantly larger eyes than WL chicks.

Body weight

At all ages, BR chicks were heavier than WL chicks although the difference was not significant till week 2 (Fig. 2.1.4). The difference between the weights of chicks of different breeds increased with age; by 6 weeks of age BR chicks were 3 times heavier than WL chicks. As chicks were raised in equivalent environments, the difference in body growth can be assumed to be genetically determined and is consistent with their respective uses, i.e. BR chicks are bred for meat and must be fast growing while WL chicks are egg layers and fast body growth is not as crucial. The differences in eye size mentioned previously are a reflection of the differences in body size, with large chicks having large eyes and smaller chicks having smaller eyes. Eye volume was significantly correlated to body weight for both groups; $r = 0.997$ ($P < 0.005$) for WL chicks and $r = 0.970$ ($P < 0.05$) for BR chicks (Fig. 2.1.5). The poorer correlation for the

BR chicks reflected the greater increase in body weight compared with eye volume at later ages.

Response to form deprivation: lid suture

Lid suture led to large myopic refractive errors for both breeds. Even though the axial length of normal eyes was similar for the two breeds at weeks 1 and 2, after 2 weeks of lid suture, there were significantly larger myopic shifts for WL compared with BR chicks ($P < 0.01$, MWUT; Table 2.1.1). For WLs, treated eyes were −25.4±4.8 D more myopic than contralateral normal eyes; for BR chicks, the difference was −19.6±6 D. For both breeds the ALs of lid-sutured eyes were greater than normal. Consistent with the greater myopic shift in WLs, axial changes were also greater in this breed. The difference between breeds in treatment effect on AL was statistically significant ($P < 0.005$, MWUT; weeks 1 and 2). The mean AL of sutured eyes of WL chicks was 1.15±0.16 mm (13.6% increase compared with normal AL) and 2.06±0.18 mm (22.2% increase) greater than that of normal eyes after 1 and 2 weeks deprivation respectively; the equivalent values for BRs were 0.69±0.22 mm (8.1% increase) and 1.55±0.38 mm (16.7% increase) at 1 and 2 weeks.

Vitreous chamber growth accounted for 90%, i.e. 1.04±0.06 mm (20.8% increase compared with normal eyes), of the axial change for WLs after 1 weeks lid suture. In contrast for BR chicks, the increase in the VCD, i.e. 0.76±0.11 mm, (14.5% increase) was greater than the measured increase in AL. Vitreous chamber expansion was even greater after two weeks of lid suture, although it contributed less to the overall axial change, with 1.55±0.12 mm (75% ΔAL; 28.5% increase compared with normal) and 1.23±0.26 mm (80% ΔAL; 21.9% increase) increase in VCD for WL and BR groups respectively. The discrepancy between changes in AL and VCD were largely due to changes in the anterior chamber. Although the anterior chamber response to lid suture was initially slower than that for the vitreous chamber, the percentage increases in ACD were greater than those for VCD after 2 weeks deprivation. Lid suture caused deepening of the anterior chamber for the WL group after both 1 and 2 weeks of deprivation; 0.1±0.1 mm (10% ΔAL; 7.9% increase compared with normal eyes) at week 1 and 0.51±0.16 mm (25% ΔAL; 35% increase) at week 2. For the BR group the anterior chamber was much slower to respond to deprivation; deepening was only seen after 2 weeks, i.e. 0.35±0.38 mm (20% ΔAL; 23.2% increase); in contrast at week 1, anterior

chamber shallowing of 0.09±0.1 mm (7.0% decrease) was measured. This difference in treatment effect between the two breeds on the anterior chamber was significant at both time points ($P < 0.05$, MWUT). Differences between treated and normal eyes lens thickness were minimal for both breeds following 1 and 2 week lid suture and no consistent effect was seen.

Table 2.1.1. Differences, in ocular parameters, between treated eyes and normal eyes, after 1 and 2 weeks lid suture (mean ± SD, n = 8, 6).

Ocular parameter	Week 1		Week 2	
	WL	BR	WL	BR
Δ Refraction (D)	–	–	–25.4±4.8**	–19.6±6
Δ Corneal power (D)	–	–	+3.9±4.0*	–1.7±2.4
Δ Anterior chamber depth (mm)	+0.10±0.1**	–0.09±0.1	+0.51±0.16**	+0.35±0.38
Δ Axial lens thickness (mm)	+0.01±0.03	+0.02±0.03	–0.01±0.03	–0.02±0.03
Δ Vitreous chamber depth (mm)	+1.04±0.12***	+0.76±0.22	+1.55±0.12**	+1.23±0.26
Δ Axial length (mm)	+1.15±0.16***	+0.69±0.22	+2.06±0.18***	+1.55±0.38

*Differences between White Leghorn chicks and broiler chicks significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).*

Corneal steepening of 3.9±4.0 D was observed at week 2 for lid-sutured WL chicks. In contrast, corneal flattening of 1.7±2.4 D was observed for the lid-sutured BR group. The difference in corneal response to lid suture between WL and BR chicks was statistically significant ($P < 0.05$, MWUT)

Response to form deprivation: occlusion

Greater myopic shifts were also produced by occlusion for WL compared with BR chicks, i.e. –31.0±4.8 D compared with –21.3±8.5 D for WL and BR

chicks respectively at week 2 (Table 2.1.2; P < 0.005, MWUT). Consistent with the greater myopic response occlusion produced greater axial elongation in WL chicks compared with BR chicks, i.e. 1.80±0.37 mm (19.6% increase compared with normal) compared with 1.22±0.6 mm (13.0% increase) respectively (P < 0.005, MWUT).

Table 2.1.2. Differences, in ocular parameters, between treated eyes and normal eyes after 2 weeks monocular occlusion (mean ± SD, n = 8, 6).

Ocular parameter	Week 2	
	WL	BR
Δ Refraction (D)	−31.0±4.8***	−21.3±8.5
Δ Corneal power (D)	+4.8±4.2	+2.8±5.4
Δ Anterior chamber depth (mm)	+0.63±0.24***	+0.39±0.28
Δ Axial lens thickness (mm)	+0.01±0.02	−0.01±0.03
Δ Vitreous chamber depth (mm)	+1.16±0.38***	+0.85±0.11
Δ Axial length (mm)	+1.80±0.37***	+1.22±0.22

*Differences between White Leghorn chicks and broiler chicks significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).*

The greater axial elongation in WL chicks was due to both greater vitreous and anterior chamber elongation. The ACD changes, i.e. 0.63±0.24 mm (43.4% increase compared with normal eye) and 0.39±0.28 mm (27% increase) for WL and BR chicks respectively at week 2, contributed 35% and 30% respectively to the axial changes. While the difference in magnitude of response of occlusion on the anterior chamber was significantly different for the two breeds (P < 0.005, MWUT), the relative contribution that the anterior chamber changes made to the axial changes were similar. Vitreous chamber changes of 1.16±0.38 mm (21% increase) and 0.85±0.11 mm (14.7% increase) for WL and BR chicks respectively, contributed 65% and 70% to the axial changes produced by occlusion. While the magnitude of response was significantly different between breeds (P < 0.005, MWUT), the contribution the vitreous chamber elongation made to the axial changes was similar. Corneal steepening of similar magnitude was observed for both breeds in

response to occlusion, i.e 4.8±4.2 D and 2.8±5.4 D for WL and B respectively. Although not significantly greater, the trend towards greater steepening in response to occlusion for WL chicks is consistent with the greater anterior chamber deepening observed in this breed. There was no significant effect of occlusion on the measured lens thickness for either breed.

Occlusion produced greater myopic shifts than lid suture for both breeds, i.e. 22% increase in myopia for WL chicks and 5.5% for BR chicks; variations between chicks were also greater. Although greater myopia was produced by occlusion compared with lid suture for both breeds, the difference was only significant for WL chicks ($P < 0.05$, MWUT). In contrast to the greater myopia produced following two weeks of occlusion, the measured axial change was significantly less and also more variable than that produced by the equivalent duration of lid suture for both breeds, i.e. 14.4% less axial elongation for WL chicks ($P < 0.05$, MWUT) and 27% for BR chicks ($P < 0.01$, MWUT). In parallel with the axial changes, vitreous chamber changes were less when the deprivation was produced by occlusion, occlusion only producing 25.2% and 30.8% of the effect on the vitreous chamber seen with lid suture for WL and BR chicks respectively. In contrast, greater anterior chamber deepening resulted from 2 weeks occlusion compared with the equivalent period of lid suture; 17.6% and 11.4% greater anterior chamber deepening occurred for the WL and BR groups respectively. Thus, changes in the ACD contributed more to the AL changes for occlusion compared with lid suture, i.e. 35% compared with 25% and 30% compared with 20% for WL and BR groups respectively. As changes in ACD produce greater changes in refraction than changes in VCD this could account for the greater myopia seen. Consistent with the anterior chamber effect, corneal steepening produced by occlusion, although not significant, was greater for both breeds than that produced by lid suture.

Recovery from form-deprivation myopia

Form-deprivation myopia decreased quickly once normal vision was restored. This occurred regardless of breed or whether the myopia was produced by lid suture (Fig 2.1.6) or occlusion (Fig 2.1.9). However recovery was much more rapid for BR chicks, 2 weeks of normal vision being required to reduce the refractive error of previously lid-sutured eyes to that of normal eyes, i.e. –0.1±1.3 D for treated eyes compared with

+0.1±0.5 D for normal eyes. For BR chicks, the greatest reduction in myopia occurred during the first week of normal vision with the mean refraction of treated eyes being 13.8 D less myopic than at eye opening. Refractive recovery was slower in WL chicks, with the refractive error of treated eyes not normalizing until week 5, after 3 weeks of normal vision. Recovery was also slower in onset, the reduction in myopia of previously lid-sutured eyes of WL chicks being greatest during the second week of normal vision. A 7.5 D reduction in myopia was seen in the first week compared with a 13.5 D decrease during the second. Consistent with the breed difference in recovery rates, interocular differences in refraction were statistically greater for the WL group compared with the BR group at weeks 2, 3 and 4. Similar trends were observed for recovery following occlusion with statistically greater residual myopia being observed in the WL group at the same time points. However, in contrast to the slower onset of recovery from lid suture, recovery of occluded WL chicks was similar to BR chicks with fastest recovery rates during the first week of normal vision.

During recovery, there was initially total inhibition of ocular growth and then slowed ocular growth relative to normal growth until treated eyes had normalized with respect to their axial dimensions. Previously lid-sutured eyes of BR chicks had normal axial dimensions by week 4, after 2 weeks of normal vision, with refractive errors returning to normal values over the same period (Fig. 2.1.6). Recovery of normal axial dimensions was slower for sutured WL chicks. This did not occur until week 5, consistent with a slower refractive recovery. Treated eyes of BR chicks made myopic by occlusion had normal axial dimensions by week 4; again recovery for WL chicks was slower, with normal values not being attained until week 6 (Fig 2.1.9).

The differences in recovery of AL between breeds was due to differential recovery of the anterior and vitreous chambers. ACD recovery was much slower and less complete for WL compared with BR chicks. For BR chicks the anterior chamber was deepened by 2 weeks of lid suture; the ACDs of treated and normal eyes were the same by week 5. During the first week of normal vision, the difference between treated and normal eyes of BR chicks remained the same. Subsequently the anterior chamber growth of the previously lid-sutured eye slowed and the difference between the ACDs of treated and normal eyes lessened (Fig. 2.1.7). This pattern contrasted with the response of WL chicks where, during the first week of normal vision, the anterior chamber of treated

eyes actually continued to deepen at a greater rate than normal; it was not until the second week that the growth of the anterior chamber was slowed relative to normal and the difference between treated and normal eyes decreased. However, the ACD of the treated eye of WL chicks did not return to normal values by the last measurement point, i.e. it continued to be deeper than normal in contrast to BR chicks were anterior chamber normalization occurred. Similar results for ACD were obtained for recovery from occlusion; for BR chicks, ACDs of recovery eyes were equivalent to those of normal eyes at week 4, while the ACD of treated eyes of WL chicks remained deeper than normal throughout the monitoring period (Fig 2.1.10).

Vitreous chamber growth of treated eyes of both breeds was inhibited during recovery; unlike the anterior chamber response, the difference between the VCD of treated and normal eyes immediately began to decrease once vision was restored. VCDs of treated eyes normalized by week 4 for previously lid-sutured BR chicks, by week 5 for previously lid-sutured WL chicks and by week 4 for both occlusion groups. This is again in contrast to the anterior chamber where variable recovery was observed. Lenses in recovery eyes tended to be slightly thinner than normal for all groups.

Corneal steepening resulted from both lid suture and occlusion in WL chicks and this corneal steepening was not affected by restoration of normal vision. In contrast, corneal steepening observed in occluded BR chicks normalized by week 3.

Growth rates

Although emmetropia was achieved for both breeds following restoration of normal vision, the ocular components recovery for the treated eyes of the two breeds were significantly different. These differences were most likely to be a reflection of the magnitude of the changes in ocular growth produced by visual deprivation and the magnitude of normal ocular growth that occurred during the period of recovery (Fig. 2.1.12; see Appendix II, Tables AII.2.1, for treated and normal eye data). BR chicks, being less responsive to deprivation and having faster normal growth during the recovery period, were able to normalize refraction and ocular dimensions more rapidly than WL chicks.

The breeds mainly differed in the recovery of the anterior segment following form deprivation. For ACD the differences between breeds were a reflection of the differences in magnitude of the ACD changes produced by deprivation and differences in normal ACD growth during the period of recovery. For WLs, lid suture produced a 35% increase in ACD, the ACD continued to increase at a greater rate than normal so that by week 3 a 44.7% increase was observed and the mean ACD of treated eyes was 2.33 mm at this time. However from weeks 3 to 6, the ACD of normal eyes only increased 25%, i.e. to 2.02 mm, and thus at 6 weeks of age, the ACD of treated eyes was still 0.18 mm greater than normal. For BR chicks, 2 weeks lid suture resulted in a smaller increase in ACD, i.e. 23.2%, although here also the difference between treated and normal eyes was greater at week 3 due to the ACD of the normal eye also increasing. However the percentage increase was less, i.e. 21.8%; the peak ACD of treated eyes, at week 3, was 2.07 mm. From weeks 3 to 6, the ACD of normal eyes of BR chicks increased by 34.7%, i.e. to 2.29 mm, as normal growth is greater than the change produced by deprivation the ACD of treated eyes eventually normalized.

Differences also occurred in recovery of the vitreous chamber, with the VCD of BR chicks normalizing faster. Lid suture in WL chicks produced a mean 28.5% increase in the depth of the vitreous chamber but from weeks 2 to 6 the VCD of normal eyes only increased 25.5%. The VCD of WL chicks on lid opening was 6.99±0.17 mm, the VCD then appeared to "shrink" to 6.51±0.15 at week 3, i.e. a mean decrease of 0.49±0.3 mm in length and continued to "shrink" until week 5 when a VCD of 6.36±0.22 mm was measured. The VCD then increased over the remaining week to a mean value of 6.66±0.21 mm at week 6, which is slightly shorter than the value 6.83±0.17 mm for normal eyes. In contrast, for BR chicks, VCD only increased by 21.9% with lid suture and from weeks 2 to 6, VCD of normal eyes increased 31.0%. The mean VCD of BR chicks on lid opening was 6.85±0.17 mm; as with WLs, the VCD then appeared to "shrink" to 6.33±0.11 mm at week 3 before again increasing to a final value of 7.54±0.18 mm at week 6. The equivalent value for normal eyes was 7.36±0.19 mm.

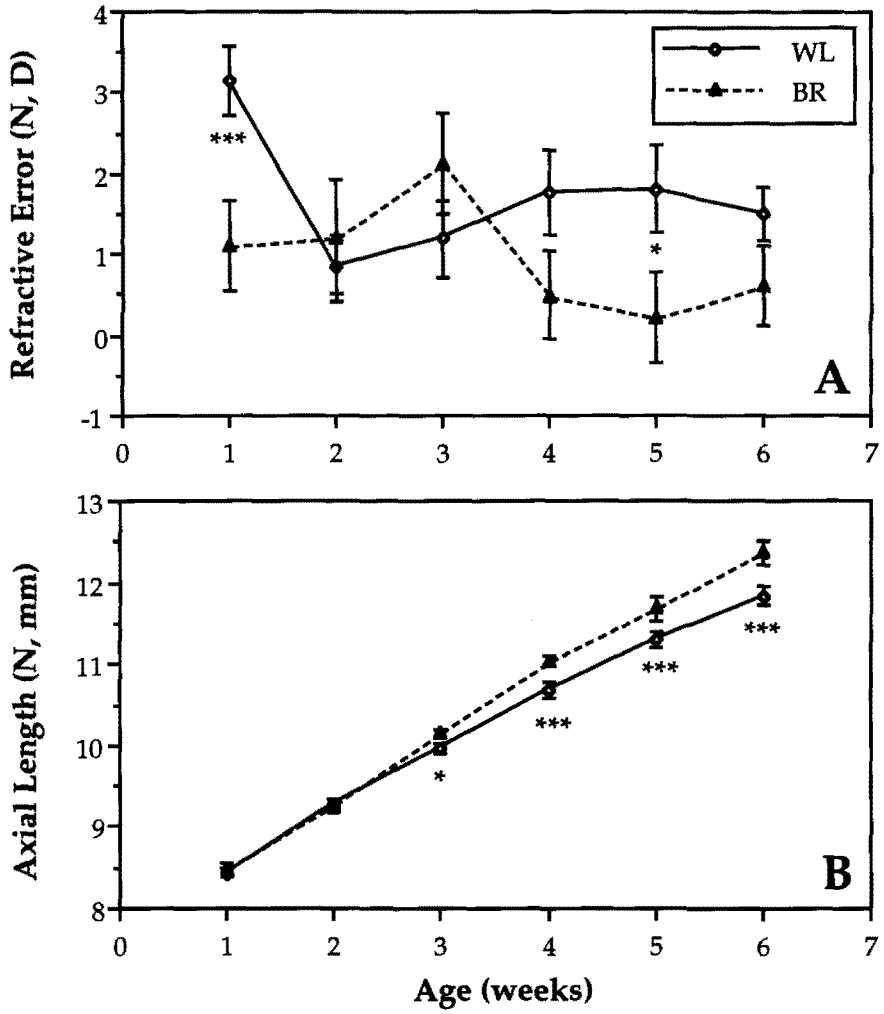


Figure 2.1.1. **A.** refractive error and, **B.** axial length (mean ± SE) of normal (N) eyes of White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

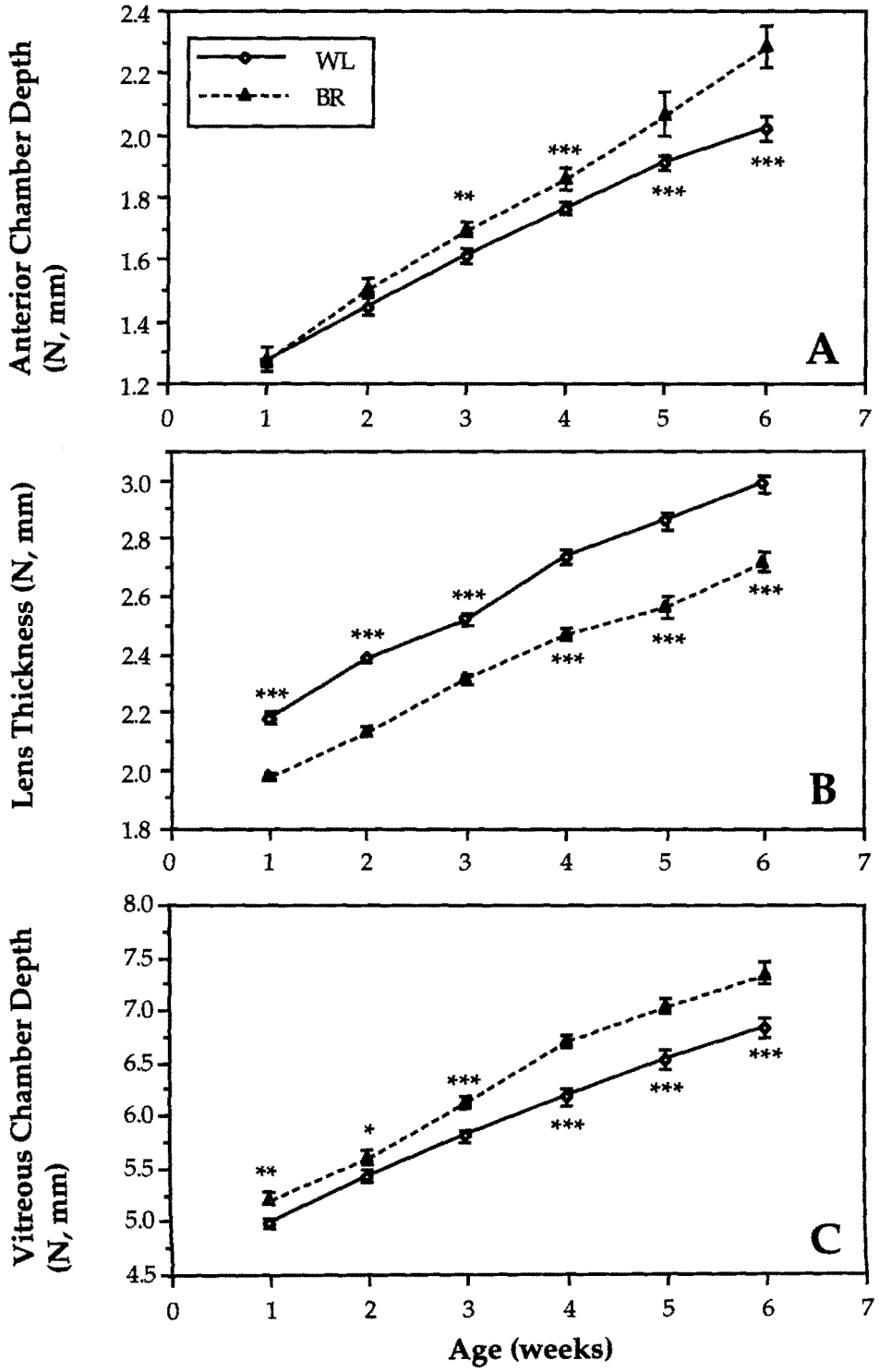


Figure 2.1.2. **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth (mean ± SE) of normal (N) eyes of White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

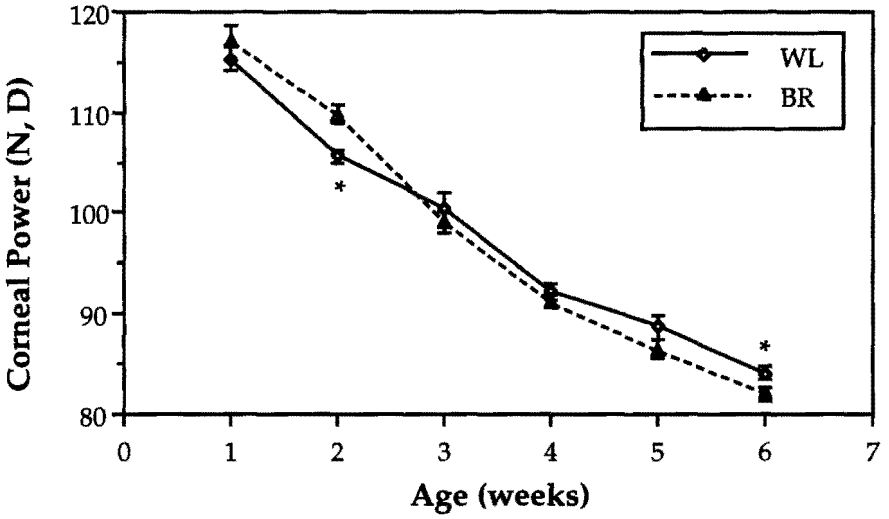


Figure 2.1.3. Corneal power (mean ± SE) of normal (N) eyes of White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

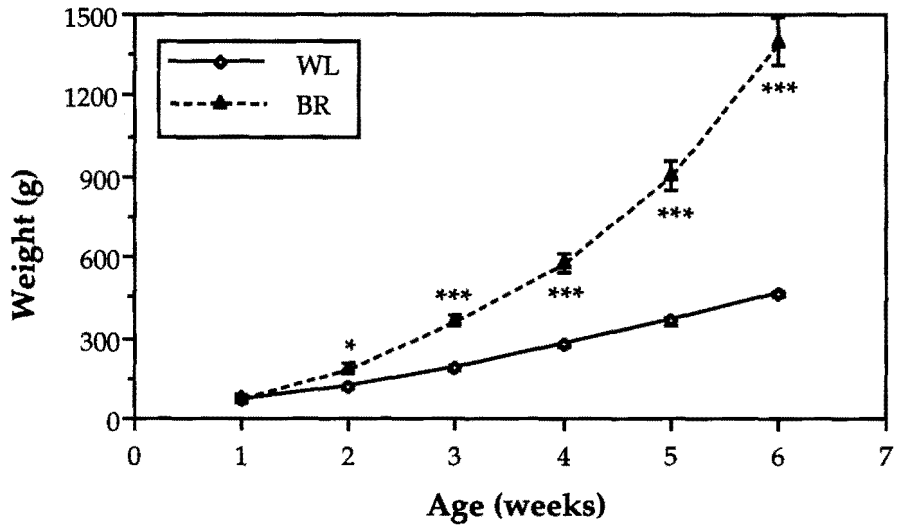


Figure 2.1.4. Body weights (mean ± SE) for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

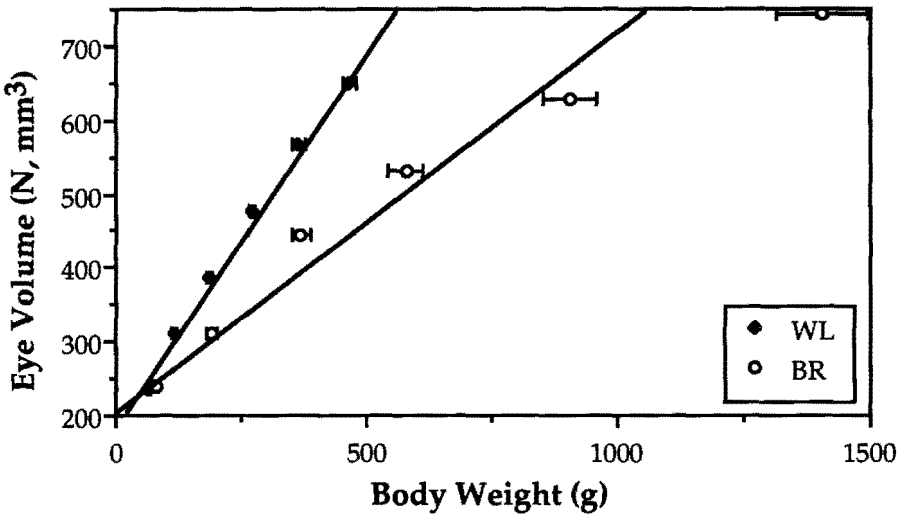


Figure 2.1.5. Correlation between normal eye volume and body weights (mean ± SE) for White Leghorn (WL) and broiler (BR) chicks. Eye weight was significantly correlated to body weight; r =0.997 ($P < 0.005$) for WL chicks and r =0.970 ($P < 0.05$) for BR chicks.

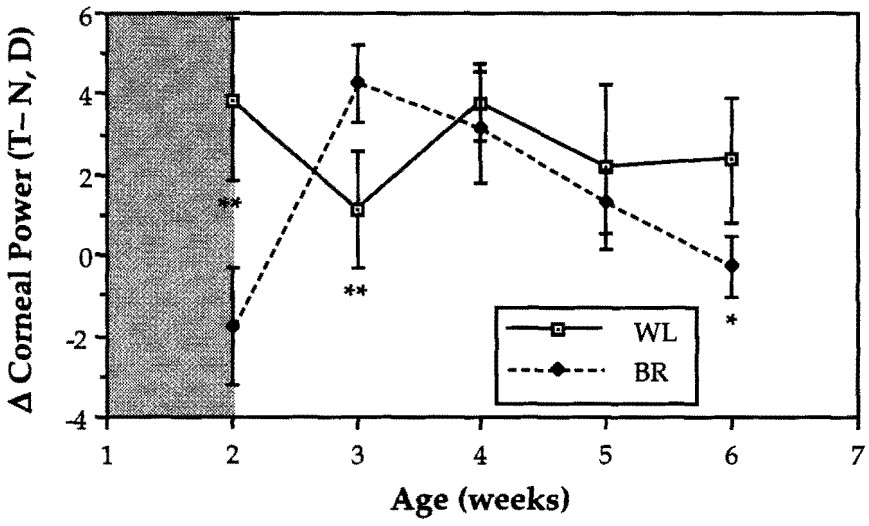


Figure 2.1.8. Differences in corneal power (mean ± SE) between treated (T) and normal (N) eyes for 2 weeks lid suture (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, Mann-Whitney U-test (two-tailed).

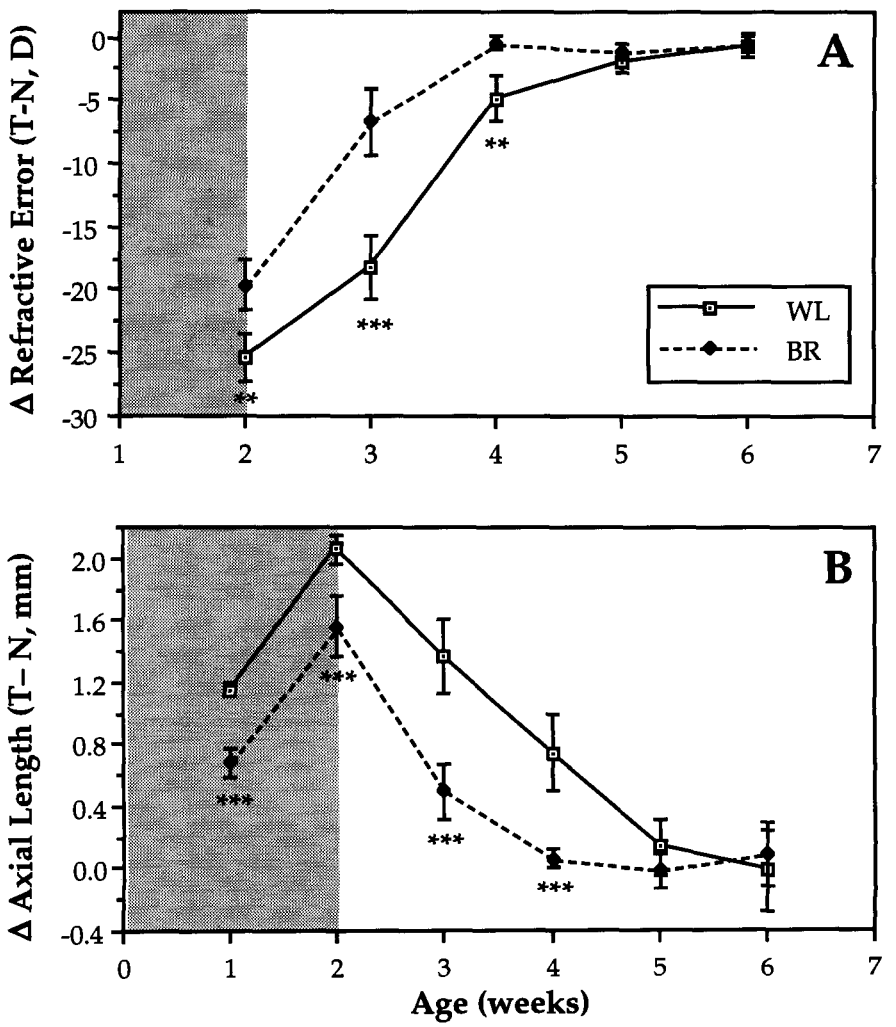


Figure 2.1.6. Differences (mean ± SE) in **A.** refractive error and, **B.** axial length between treated (T) and normal (N) eyes for 2 weeks lid suture (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

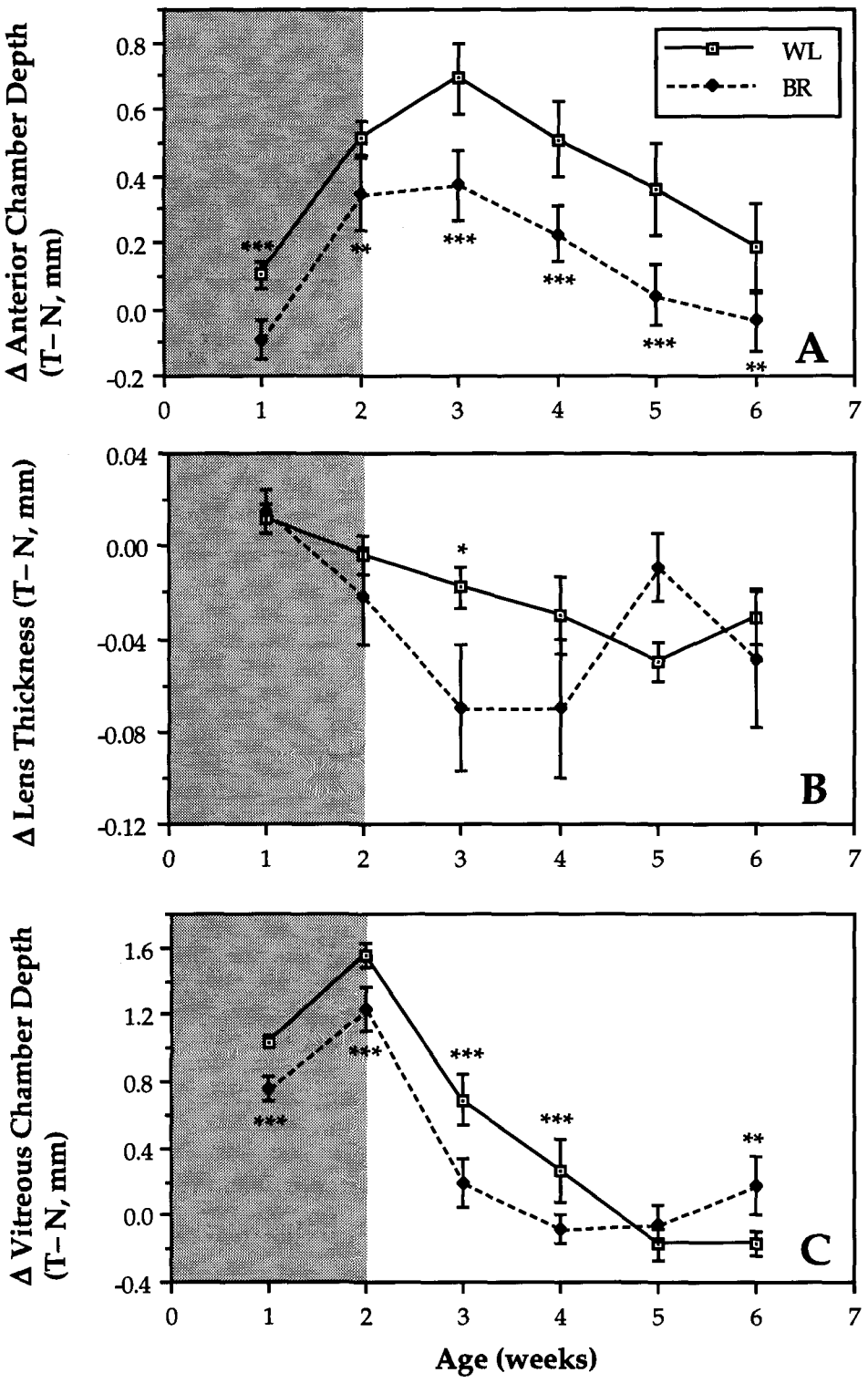


Figure 2.1.7. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes for 2 weeks lid suture (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

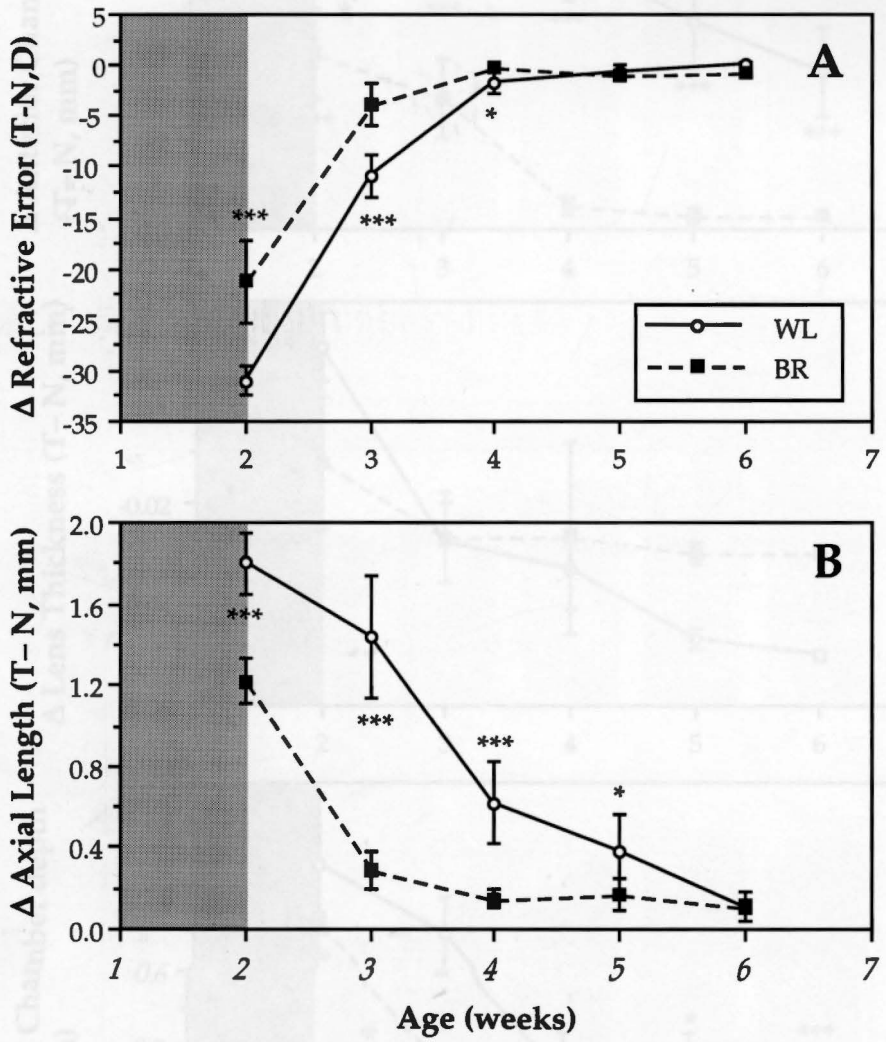


Figure 2.1.9. Differences (mean ± SE) in **A.** refractive error and, **B.** axial length between treated (T) and normal (N) eyes for 2 weeks occlusion (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.005, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

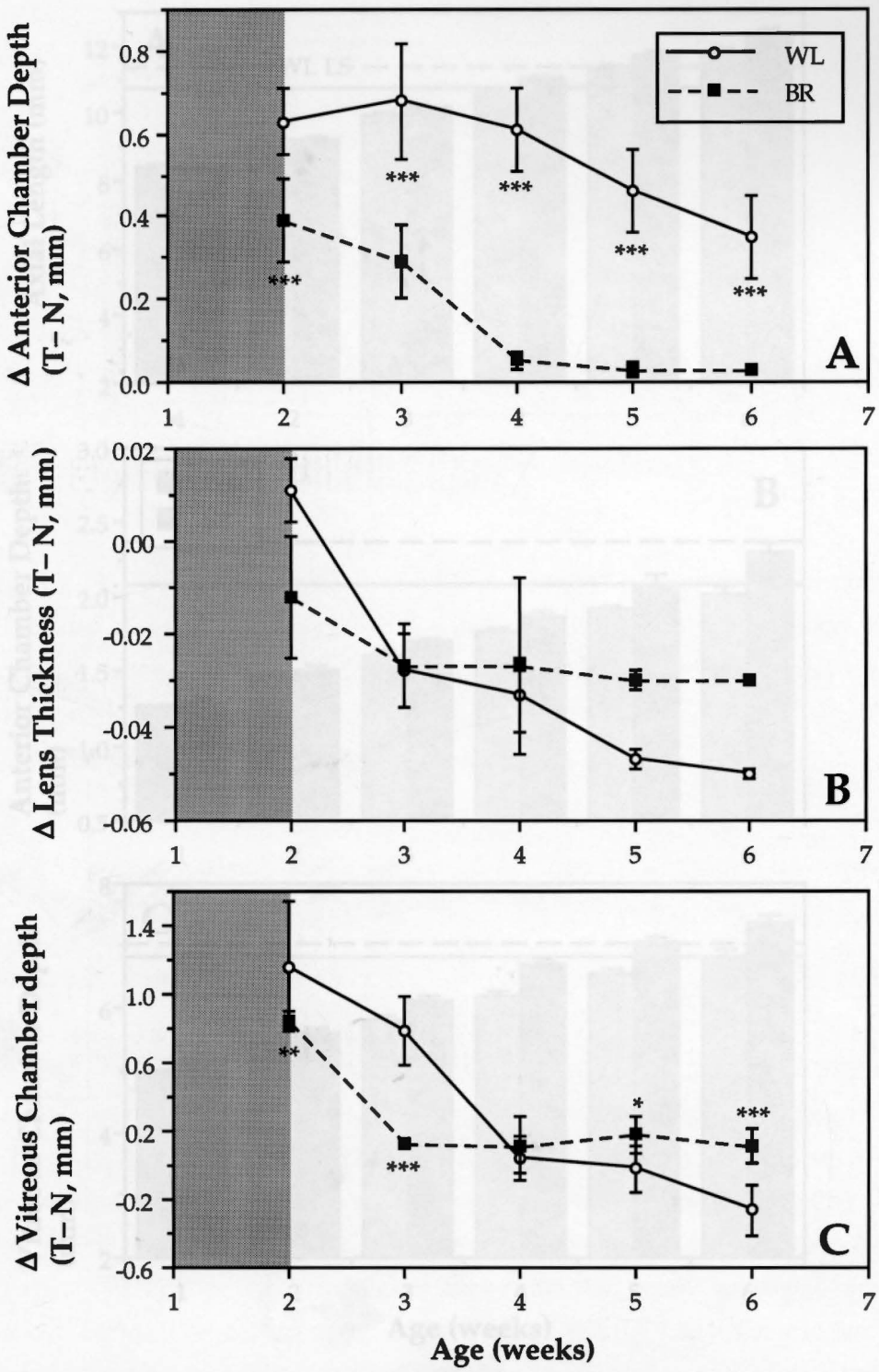


Figure 2.1.10. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes for 2 weeks occlusion (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

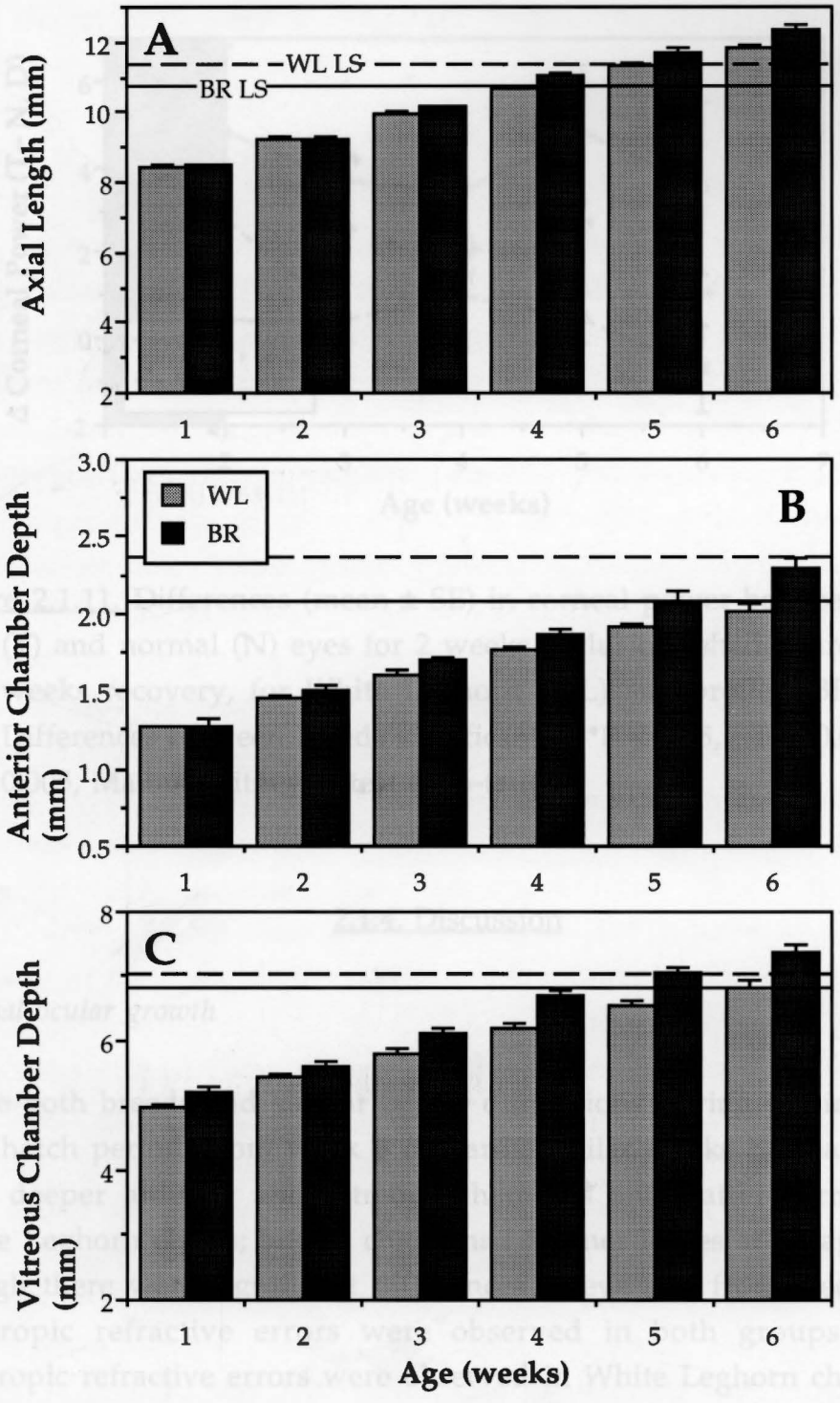


Figure 2.1.12. Comparison of lid-suture effect on treated eyes to normal growth for **A.** axial length, **B.** anterior chamber depth and **C.** vitreous chamber depth for White Leghorn (WL) and broiler (BR) chicks. Normal ocular growth is plotted as a function of age. The horizontal line indicates the treated eye dimensions following 2 weeks of lid suture, for AL and VCD and following 2 weeks of lid suture and 1 week of normal vision for ACD.

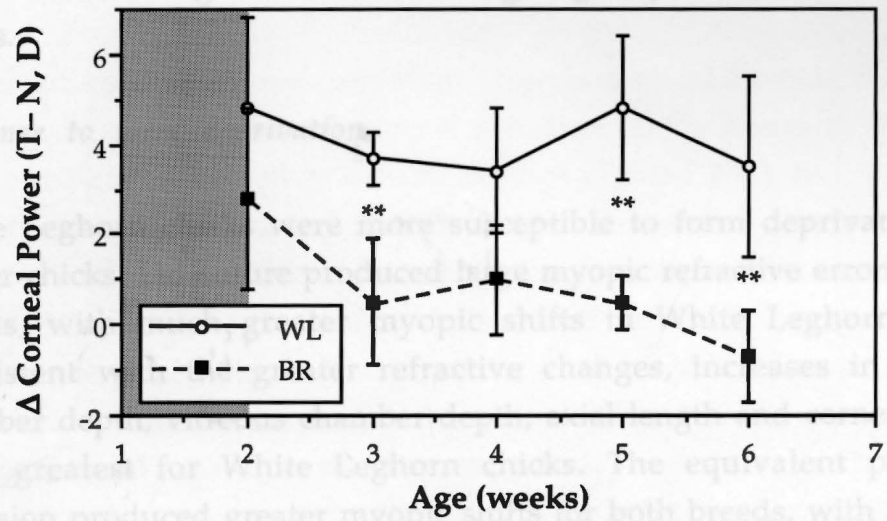


Figure 2.1.11. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes for 2 weeks occlusion (shaded area) and 4 weeks recovery, for White Leghorn (WL) and broiler (BR) chicks. Differences between breeds significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

2.1.4. Discussion

Normal ocular growth

While both breeds had similar ocular dimensions during the immediate post hatch period, from week 3 onwards broiler chicks had larger eyes with deeper anterior and vitreous chambers and flatter corneas than White Leghorn chicks; broiler chicks had thinner lenses at all ages. Even though there were significant differences in eye size from week 3, low hyperopic refractive errors were observed in both groups; greater hyperopic refractive errors were observed in White Leghorn chicks only at week 1. This is consistent with "appropriate correlation" of the various ocular components for both breeds, i.e. in the case of broiler chicks the deeper anterior chambers and vitreous chambers were negated by flatter corneas and thinner lenses. Hofstetter (1967) suggested that the eye would be emmetropic "whether for a mouse or an elephant", i.e. emmetropia is independent of eye size. In this example here, the overall differences in eye size are most likely a reflection of the differences in body size, with

broiler chicks being heavier and having larger eyes than White Leghorn chicks.

Response to form deprivation

White Leghorn chicks were more susceptible to form deprivation than broiler chicks. Lid suture produced large myopic refractive errors in both breeds, with much greater myopic shifts in White Leghorn chicks. Consistent with the greater refractive changes, increases in anterior chamber depth, vitreous chamber depth, axial length and corneal power were greatest for White Leghorn chicks. The equivalent period of occlusion produced greater myopic shifts for both breeds, with a greater anterior effect and a lesser posterior effect, i.e. vitreous chamber changes; greater increases in anterior chamber depth and corneal steepening accounted for the greater myopia observed with this method of visual deprivation. This association of lid-suture with corneal flattening, suggests that the closed lids have a mechanical action on the front of the eye.

When comparing the effects of monocular and bilateral deprivation Sivak *et al.* (1989) also noted that there was a large variation in response depending on breed. Recently Troilo *et al.* (1992) have suggested a difference in the eye growth response to form deprivation of two strains of chicks of the one breed, i.e. Cornell K compared with Washington H & N White Leghorns. They also report differences in corneal responses to form deprivation between the strains studied, although their differences were much greater than reported here. In contrast to trends in the current study where the greatest anterior chamber and vitreous chamber changes occurred together in the same breed, Troilo *et al.* (1992) found that the Washington H & N strain, with the greater anterior chamber response had the lesser vitreous chamber elongation response to deprivation.

In contrast to the deprivation effects reported here, it has been suggested that White Leghorn chicks develop continuous light-induced avian glaucoma more slowly than do broiler chicks (Lauber and Kinnear, 1979; Lauber and Oishi, 1987). As both form deprivation and continuous light cause enlarged eyes these results appear inconsistent. The hypothesis that susceptibility to both form deprivation and continuous light is determined by the rate of eye growth during the period of visual manipulation thus appears to be too simplistic.

Comparison with other deprivation studies

The magnitude of form-deprivation myopia and interocular changes in dimensions as a function of normal eye dimensions found in this study were compared with other similar studies (Tables 2.1.3 and 2.1.4). The small number of comparison studies and hence breeds is due to two main factors. Firstly, it is difficult to quantitatively compare the results found here to other studies, for while studies vary in breed they also tend to vary in duration, timing and method of deprivation production. Secondly, studies which could be compared often do not report the breed of chick used. Studies were grouped depending on whether the deprivation was produced by lid suture (Table 2.1.3) or occlusion (Table 2.1.4).

While comparisons to other lid-suture groups show similarity of anterior chamber and axial changes, deprivation was performed for longer periods in the these studies. Osol *et al.* (1986) obtained results following 7 weeks of lid suture and Yinon *et al.* (1980) following 3 months. The lesser myopia in Yinon *et al.*'s (1980) study can be explained by this age difference, the same percentage changes in axial length produce less refractive effect in larger eyes.

Occlusion studies usually report less myopia than that found in the current study. The lesser response of White Leghorn to 2 weeks occlusion reported by Troilo and Wallman (1991) may be due to the use of partial rather than full occluders. Sivak *et al.* (1989a) studied the effects of monocular and binocular 2 week occlusion in White Leghorn, broiler and Rock Hen chicks. Unfortunately comparisons with the Rock Hens could not be made as they were deprived using opaque occluders. Sivak *et al.* (1989a) reports less form-deprivation myopia in White Leghorns than found here but values for broiler chicks were similar. Sivak *et al.* (1989a) suggest that the greater myopia for their broiler chicks was due to the use of bilateral as opposed to monocular deprivation. The most similar White Leghorn value to that of the current study is that obtained by Wallman and Adams (1987) who reported 26 D of myopia production following deprivation of the frontal visual field for two weeks. Hayes *et al.* (1986) reported less myopia for White Rock-Rhode Island Red chicks following 3 to 8 weeks occlusion than for either of the breeds used in this study.

The main point from these comparisons is that not only do different eye growth responses occur for different breeds of chicks, they also occur for chicks of the same breed. The differences presumably reflect slightly different experimental paradigms.

Table 2.1.3. Comparison of lid suture effects for different studies. Ocular dimension changes are reported as a function of normal eye size in percentage terms.

Ocular component	Lid suture	
	White Leghorn	Broiler
FDM (D)	–25.4, -*, –8**,	–19.6
Δ ACD (mm)	35%, 43.7%*, -**	23.2%
Δ VCD (mm)	28.5%, -*, -**	21.9%
Δ AL(mm)	22.2%, 23%*, 20%**	16.7%

*Studies quoted: Current study,*Osol et al. (1986), **Yinon et al (1980). "-" used where no value was reported.*

Table 2.1.4. Comparison of translucent occlusion effects for different studies. Ocular dimension changes are reported as a function of normal eye size in percentage terms.

Ocular component	Occlusion		
	White Leghorn	Broiler	White Rock-Rhode Island Red
FDM (D)	–31, –11.3*, –15.3 •, –26**	–21.3, –17.8•	–14.9••
Δ ACD (mm)	43.4%, 0%*, -•, -**	27%, -•	33%••
Δ VCD (mm)	21%, 9.7%*, -•, -**	14.7%, -•	11.8%••
Δ AL(mm)	19.6%, -*, -•, -**	13%, -•	15.5%••

*Studies quoted: Current study,*Troilo and Wallman (1991), •Sivak et al. (1989a), **Wallman and Adams (1987), ••Hayes et al. (1986). "-" used where no value was reported.*

Recovery

Regardless of the breed of chick and method of production of form deprivation, myopia decreased quickly once normal vision was restored. For the White Leghorn chicks, refractive recovery was slower than that of broiler chicks. Although emmetropia was obtained for both breeds, the two breeds differed in their rates of recovery and the final contribution of the various ocular components to the axial length. These differences are a reflection of the magnitude of the changes in ocular growth produced by form deprivation, with greater deprivation responses for White Leghorn chicks, and the magnitude of normal ocular growth during the period of recovery, with greater normal growth for broiler chicks (Fig. 2.1.12). Of the monitored ocular dimensions, recovery of the anterior chamber was poorest.

Relevance to future experiments

This investigation revealed fundamental differences in normal growth rates, magnitude of form-deprivation myopia and speed of recovery between the two breeds of chicks studied. Thus comparisons of the results of ocular growth studies involving different breeds must be done with caution and overall growth trends rather then actual quantitative data compared. To reduce breed-dependent variability, only White Leghorn or White Leghorn-New Hampshire chicks were used in the remaining experiments. Initially White Leghorn chicks were used, the change to cross-breed chicks being necessitated by closure of the original supplier.

2.1.5. Conclusion

In conclusion, different breeds of chicks do differ in their normal ocular growth, susceptibility to deprivation and speed of recovery. Despite such differences, both breeds became myopic in response to visual deprivation and showed an emmetropization growth response. The effect of breed should be considered when directly comparing the results of different experiments in the field of chicken myopia research.

2.2. The Significance of Sex of Chick

2.2.0. Summary

The sex dependence of chick eye growth and response to visual deprivation was investigated. Day-old White Leghorn chicks, both female and male, had one eye sutured closed for two weeks; eye growth was monitored weekly for 8 weeks. From 7 weeks of age onwards cockerels had significantly larger eyes than pullets, cockerels having significantly longer vitreous chambers, and slightly deeper anterior chambers. Axial length was highly correlated to body weight. Responses to form deprivation were similar for both groups; high myopia and axial elongation was observed. There were no significant differences in the magnitude of the form-deprivation response. Although the degree of form-deprivation myopia was similar, the speed of refractive and axial recovery was faster for pullets. The differences in eye growth and response to form deprivation between sexes were not great, but there are some subtle differences which should be considered for eye-growth studies.

2.2.1. Introduction

In humans, there are sex-linked differences in eye growth, with vitreous chamber depth (Larsen, 1971a) and axial length (Larsen, 1971b) being longer in males. It has also been suggested that myopia progression rates differ between male and female children. Myopia tends to develop slightly earlier in females than in males and tends to be slightly greater degree (Goss and Winkler, 1983; Goss, 1987).

Sex-linked effects on eye growth in chicks have not been investigated, even though there are significant sex differences in visual projections and visual behaviour. Asymmetry of the thalamofugal visual projections (projections fed by the right eye to the left side of the thalamus are more numerous than those fed from the left eye to the right side of the thalamus), has been reported to exist only in young male chicks (Adret and Rogers, 1989). Recently, however, this asymmetry has also been found to exist in female chicks but to a much lesser degree than in males (Rajendra and Rogers, 1993). Studies have also shown that visually-driven, lateralized behaviour is less pronounced in female

chicks compared with male chicks (Zappia and Rogers, 1987). Despite the lack of asymmetry in females on some tasks (e.g. fear response to a visual stimulus) asymmetry has been reported for other tasks (e.g. attack and copulation behaviours) (Rogers, 1982). Sex differences in secretion of growth hormone have also been reported, with male chicks showing pulsatile growth hormone secretion until a later age than female chicks. It has been suggested that the difference in growth rates of female and male chicks may be due to this difference in growth hormone (Johnson, 1988). As female and male chicks show differences in overall body growth rates it may be expected that they will also show differences in eye growth rates.

While some researchers use only male chicks (Osol *et al.*, 1986) or only female chicks for investigations of eye growth, others use a random mixture of female and male chicks in their studies (Hayes *et al.*, 1986); Wallman *et al.*, 1978b; Yinon *et al.*, 1980; Yinon *et al.*, 1982/1983; Lauber and Oishi, 1987; Pickett-Seltner *et al.*, 1988). Whether normal eye growth and the response to visual deprivation are sex dependent in the chick was investigated.

2.2.2. Methods

Animals and treatments

The eye growth of both female (F; n = 17) and male (M; n = 18) White Leghorn chicks was studied. Day-old White Leghorn chicks had one eye sutured closed (LS) for two weeks from hatching. Chicks were raised in temperature controlled enclosures with food and water provided *ad libitum*. They were exposed to a 12/12 light-dark cycle, with lights on at 7 am and off at 7 pm and light intensity of 250 lux at the level of the food trough.

Measurements

Eye growth was monitored weekly for 8 weeks. Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed to determine the refractive error and the positions of the intraocular surfaces respectively. Refractive errors, anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal

curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987), under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test was used (WSRT). To assess the difference between the two sexes of chick, the Mann-Whitney U-test was used (MWUT).

2.2.3. Results

Normal ocular growth

Up to 6 weeks of age, there was no significant difference between the two groups for any of the measured ocular parameters, i.e. refractive error, corneal power, ACD, ALT, VCD or AL. Refractive error, corneal power, ACD and ALT remained similar for the duration of the study (see Appendix II, Tables AII.2.2, for normal eye data). In contrast, at both weeks 7 and 8, the ALs of cockerels were significantly greater than those of pullets, i.e. 12.22±0.38 mm compared with 11.96±0.33 mm at week 7 (M 2.2% greater than F; $P < 0.05$, MWUT), and 12.60±0.33 mm compared with 12.36±0.38 mm at week 8 (M 1.9% greater than F; $P < 0.05$, MWUT). This difference was due to male chicks having significantly longer vitreous chambers at these two ages, i.e. 7.04±0.3 mm compared with 6.89±0.26 mm (M 2.2% greater than F; $P < 0.05$, MWUT) at week 7, and 7.29±0.25 mm compared with 7.09±0.35 mm (M 2.8% greater than F; $P < 0.05$, MWUT) at week 8. Although not significant there was also a trend for the ACD of cockerels to be slightly deeper than that of the pullets, this difference increasing with age.

Form-deprivation myopia

Both female and male chicks responded similarly to form deprivation, with high myopia and axial elongation observed for both groups. There was no significant difference in the response of female and male chicks to deprivation for any of the monitored ocular parameters. The magnitude

of the myopic shift was similar for both groups, i.e. –24.5±4.4 D for female chicks compared with –22.5±5.4 D for male chicks (Table 2.2.1). Both groups showed high levels of axial elongation which increased in magnitude with increasing duration of form deprivation, i.e. increasing from week 1 to 2. In both cases, increases in VCD contributed most to the axial elongation.

Table 2.2.1. Differences in ocular parameters (mean ± SD), between treated and normal eyes, after 1 and 2 weeks lid suture (F, n = 17; M, n = 18).

	Week 1		Week 2	
Ocular parameter	**Female**	**Male**	**Female**	**Male**
Δ Refraction (D)	–	–	–24.5±4.4	–22.5±5.4
Δ Corneal power (D)	–	–	+2.5±4.0	+0.4±3.4
Δ Anterior chamber depth (mm)	–0.06±0.14	–0.08±0.18	+0.42±0.24	+0.50±0.47
Δ Axial lens thickness (mm)	+0.03±0.04	+0.03±0.02	–0.01±0.04	+0.01±0.03
Δ Vitreous chamber depth (mm)	+0.72±0.42	+0.88±0.25	+1.07±0.82	+1.16±0.93
Δ Axial length (mm)	+0.68±0.48	+0.83±0.39	+1.48±0.89	+1.66±0.99

There were no significant differences between F and M chicks at $P < 0.05$, Mann-Whitney U-test (two-tailed).

At week 1 the elongation was due entirely to the VCD, the anterior chamber was actually slightly shallower in treated compared with normal eyes for both groups. At week 2, VCD elongation contributed 72% for female chicks and 70% for male chicks to the measured axial elongation; the ACD contributed the rest of the change. Although, at week 2, male chicks showed greater ACD and VCD deepening and axial elongation than female chicks, the difference between groups was not significant. Lens thickness was unaffected by lid suture. Slight corneal steepening was

seen in treated eyes and although this was greatest for females, the difference between groups was not significant.

Recovery from myopia

Once normal vision was restored the degree of myopia rapidly decreased for both groups (Fig 2.2.1). However, significant differences in recovery were observed. The speed of recovery was greater for female chicks with treated eyes having normal refractions at week 4, following two weeks of normal vision. Refractive recovery was slower for treated eyes of male chicks, normalization not being attained until week 5, i.e. after 3 weeks of normal vision. Due to the difference in recovery rates, the interocular difference in refraction was statistically greater for male compared with female chicks at week 4 ($P < 0.01$, MWUT).

Reductions in form-deprivation myopia occurred due to slowing of the axial growth of treated eyes compared with normal (Fig. 2.2.1). However, while the ALs of treated eyes of female chicks rapidly returned to normal values, that of male chicks, although the interocular difference did decrease, remained abnormally long for the duration of the study. The interocular difference in AL was significantly greater for male chicks from weeks 5 to 8 (all ages, $P < 0.05$, MWUT). Also, male chicks showed much greater variability in the recovery of the axial elongation then did female chicks.

For both female and male chicks the rapid initial decrease observed in the interocular AL difference was due to total inhibition of vitreous chamber growth of treated eyes, i.e. the VCD of treated eyes appeared to "shrink" (Fig. 2.2.2). The apparent "shrinkage" during the first week of normal vision was greatest for female chicks; there was a measured decrease in VCD of 0.33 mm for treated eyes of female chicks but the decrease was only 0.20 mm for male chicks. The interocular difference in VCD continued to decrease during the second week of normal vision and the VCD of treated eyes of female chicks actually became slightly smaller than normal, i.e 6.14 ± 0.46 mm compared with 6.22 ± 0.20 mm. This difference then persisted for the remainder of the study. In contrast, the mean VCD of male chicks remained greater than normal, with the measured VCD of treated eyes being 7.4 ± 0.75 mm and that of normal eyes being 7.3 ± 0.25 mm at the last measurement point. As for AL data, there was a much greater variability in the recovery response of the VCD of male chicks.

Anterior chamber recovery was much slower than recovery of the vitreous chamber (Fig. 2.2.2). The interocular difference in ACD actually continued to increase during the first week of normal vision for both groups and it was not until the second week that a reduction occurred. The increase during the first week of normal vision was greater for male chicks, i.e. the ACD increased 0.48 mm for treated eyes and 0.16 mm for normal eyes of male chicks during this period, compared with equivalent values of 0.34 mm for treated and 0.16 mm for normal eyes of female chicks. The interocular difference then decreased with normal vision but never attained normal values for either group, the ACD of treated eyes remaining deeper than that of normal eyes. The interocular difference at the last measurement point was 0.16±0.39 mm for female chicks and was significantly larger, 0.59±1.1 mm, for male chicks ($P < 0.05$, MWUT).

During recovery the lens appeared to be slightly thinner in treated eyes compared with normal eyes; this difference was maintained for the duration of the study for both treatment groups (Fig. 2.2.2).

Interocular differences in corneal power corresponded to interocular differences in ACD, which increased during the first week of recovery and decreased during the second. The corneal curvature of treated eyes tended to be steeper than normal eyes at all measurement points for both groups (Fig. 2.2.3); the difference was greater for female chicks. For both groups lid suture resulted in corneal steepening, the degree of steepening increasing during the first week of normal vision and then beginning to decrease.

Body weight

Body weights of female and male chicks were only significantly different at week 8, with male chicks being heavier then female chicks ($P < 0.05$, MWUT). Although not significant this difference was also present at weeks 6 and 7 (Fig. 2.2.4).

Comparison of lid-suture effects and normal growth

Although there were no significant differences in the effect of form deprivation on the ocular growth of female and male chicks, AL, ACD and VCD changes were slightly greater for male chicks and this may account for the difference observed in recovery (Fig. 2.2.5). Two weeks of deprivation resulted in 15.9% increase in AL for female and 17.8% for

male chicks. This comprised a 29.0% increase in ACD and a 19.5% increase in VCD for female chicks; equivalent values were 33.3% and 20.9% for male chicks. The increase in the ACD continued to week 3, when percentage increases of 35.8% and 49% were observed for treated compared with normal eyes of female and male chicks respectively.

Paradoxically normal growth was slightly greater in the recovery period, i.e. between weeks 2 to 8, for male compared with female chicks and this should have aided the recovery of male chicks. The AL increased 33.2% and 34.8% during the 6 week recovery period for female and male chicks respectively; equivalent values were 29.1% and 31.1% for VCD. Normal ACD growth for 5 weeks recovery, i.e. between weeks 3 and 8, was 30.9% and 32.5% for female and male chicks respectively. As for the previous section (section 2.1), normal growth of the ACD during the recovery period was inadequate for normalization of the deprived eye. This was true for both male and female White Leghorn chicks.

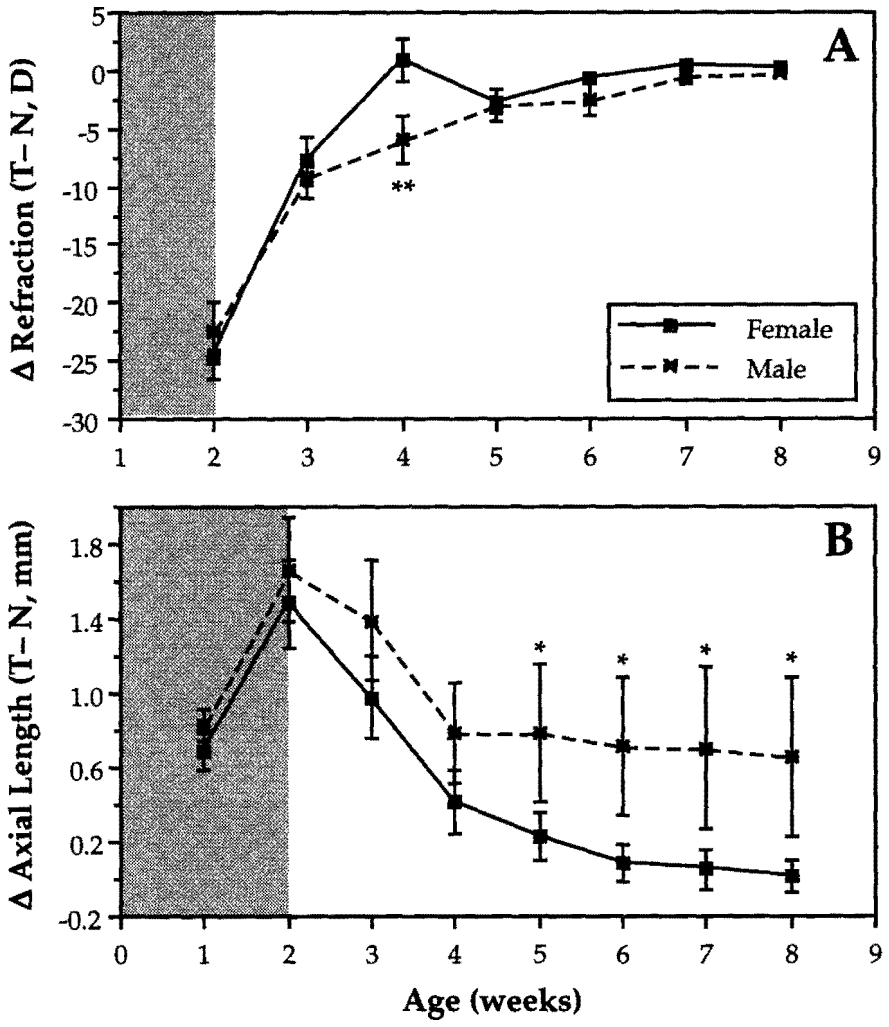


Figure 2.2.1. Differences (mean ± SE) in **A.** refraction and, **B.** axial length between treated (T) and normal (N) eyes, after two weeks lid suture (shaded area) and 6 week recovery, for female (F) and male (M) chicks Differences between sexes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

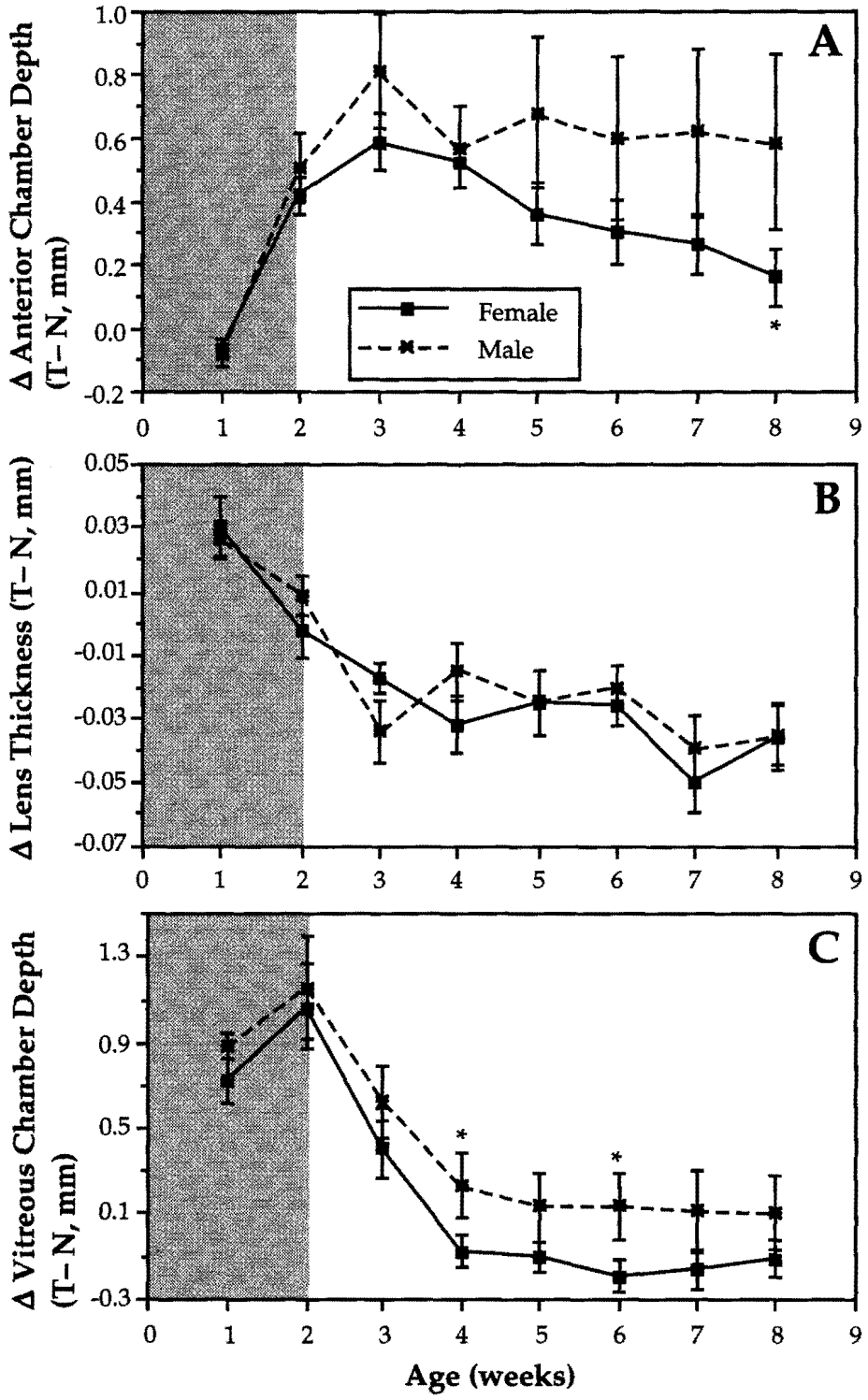


Figure 2.2.2. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after 2 weeks lid suture (shaded area) and 6 weeks recovery, for female (F) and male (M) chicks. Differences between sexes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

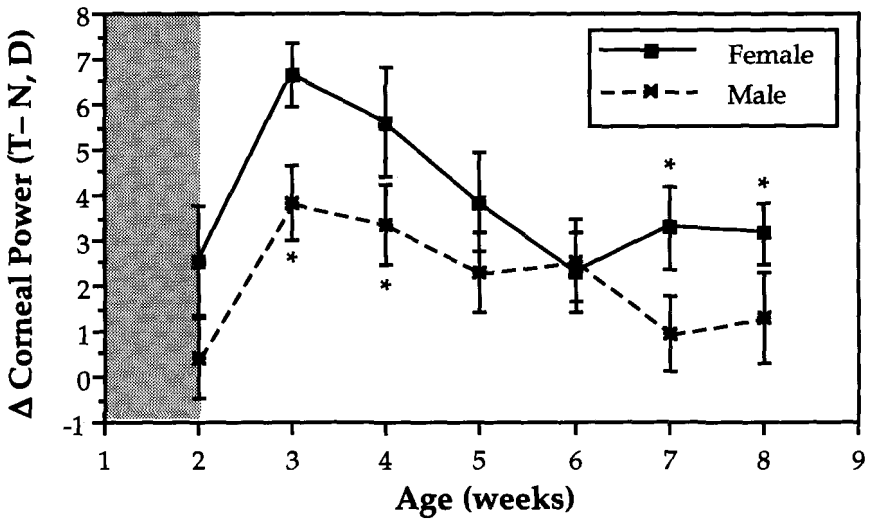


Figure 2.2.3. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes after 2 weeks lid suture (shaded area) and 6 week recovery, for female (F) and male (M) chicks. Differences between sexes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

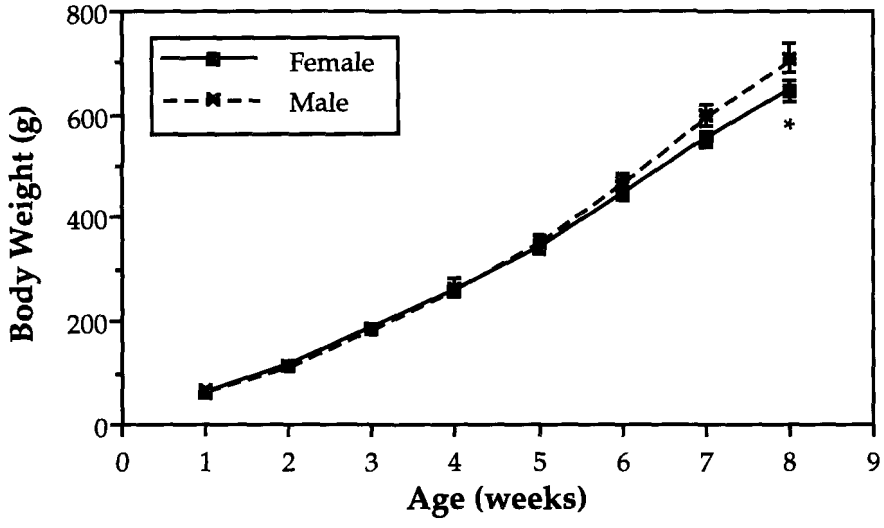


Figure 2.2.4. Body weight (mean ± SE) for female (F) and male (M) chicks. Differences between sexes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

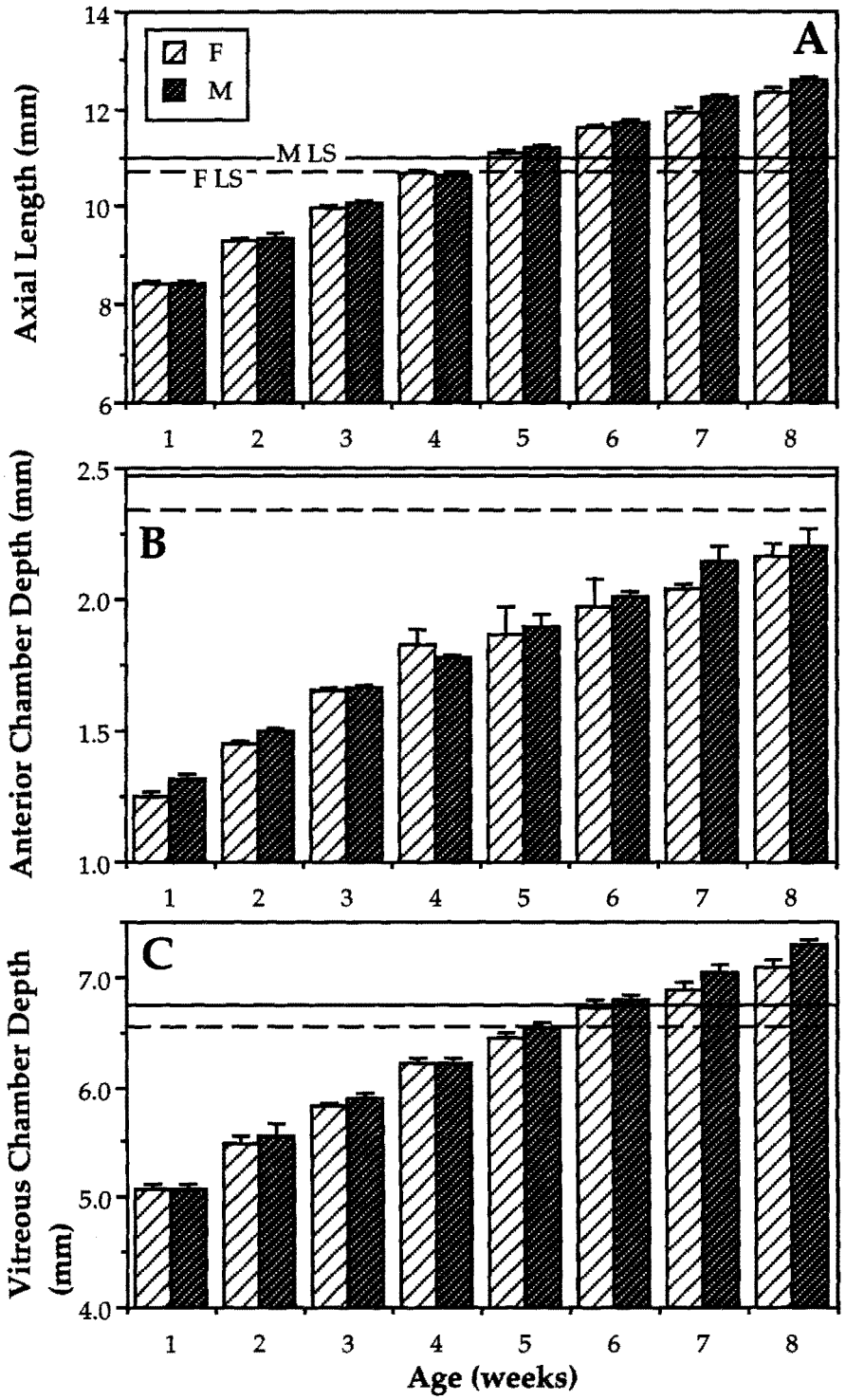


Figure 2.2.5. Comparison of lid suture response and normal growth (mean ± SE) for **A.** axial length, **B.** anterior chamber depth and **C.** vitreous chamber depth, for female (F) and male (M) chicks. For AL and VCD the horizontal line is the value for the treated eye following 2 weeks lid suture; for ACD the line represents the 3 week value, i.e. following 1 week of normal vision.

2.2.4. Discussion

Normal ocular development

From 7 weeks of age, cockerels had significantly larger eyes than pullets. This difference was primarily due to the cockerels having significantly longer vitreous chambers; there was also a tendency for the anterior chamber depth to be slightly greater. This difference was most likely due to the greater body weight of male chicks; axial length was highly correlated to body weight at both weeks 7 and 8 (both ages, $r = 0.998$, $P < 0.005$). Further evidence that ocular and body growth are linked comes from human data that reports that cessation of myopia progression in females occurs earlier than in males, presumably at the same time as reduction in body growth rate (Goss and Winkler, 1983).

Form-deprivation myopia

Female and male chicks responded similarly to form deprivation, with high myopia and axial elongation for both groups. Although effects were slightly greater for male chicks, the difference between groups was not significant. The similarity of effect is probably a reflection of the similar normal growth rates during the form deprivation period.

Recovery from form-deprivation myopia

Once normal vision was restored the magnitude of myopia rapidly decreased for both groups. However, even though the magnitude of the form-deprivation response was similar for the two sexes, the speed of axial and refractive recovery was greatest for female chicks. The mean vitreous chamber depth of treated eyes of male chicks remained slightly greater than normal for the duration of the study. Similarly, recovery of the anterior chamber was never complete for either group, but was less so for male chicks. In the previous section (section 2.1) relatively poor emmetropization was explained by both increased initial deprivation effects and decreased normal growth during recovery. However this does not explain the results here. Male chicks with the poorer emmetropization response did not exhibit greater deprivation effects and actually had slightly greater normal growth during recovery than female chicks. The only explanation is some sex-dependent effect results in

greater variability in the response of male chicks. Discussions as to whether this could be a hormonal or other physiological effect are outside the scope of this thesis.

Significance for future experiments

Although the differences in eye growth and response to deprivation between sexes are not great, there are some subtle differences. These differences should be considered and equal numbers of male and female chicks used in treatment groups to be compared. To remove one possible source of experimental variability only male chicks were used in the experiments to follow.

2.2.5. Conclusion

In conclusion, there are differences between cockerels and pullets with respect to normal eye growth and recovery from the effects of form-deprivation. These differences should be considered and equal numbers of male and female chicks used in treatment groups to be compared.

2.3. The Significance of Eye Chosen for Treatment

2.3.0. Summary

Whether the eye growth of chicks and their response to visual deprivation was affected by the eye occluded was investigated. Day-old White Leghorn chicks had either the right or the left eye lid sutured for two weeks. Eye growth was monitored weekly for 8 weeks. There was no significant difference between the normal eyes of the different treatment groups at any age. *High myopia and axial elongation resulted regardless* of which eye was deprived. Although not significant, greater myopia was produced after two weeks lid suture of the right eye; there was less myopia produced but greater variability, when the left eye was sutured. Recovery from deprivation effects occurred for both right and left treated eyes, with refractive errors of treated eyes attaining normal values for both groups by week 7. Although refractions normalized, the axial length of treated eyes remained slightly greater than normal, with greatest remaining axial elongation for left treated chicks. There were significant

differences between the groups in recovery of both the anterior and vitreous chambers. The results indicate that equal proportions of right and left treated eyes should be used in experiments, where the results of different treatments are to be compared.

2.3.1. Introduction

Many visual functions in the chick show right/left asymmetry. The right eye has been shown to be dominant for learning of visual discrimination tasks (Zappia and Rogers, 1987), while the left controls attack and copulation behaviour and learning of spatial information (Andrew, 1988; Rashid and Andrew, 1989). For example, Mench and Andrew (1986) found that male and female chicks using their right eye performed better on food grain/pebble discrimination tasks than those using the left eye (Mench and Andrew, 1986). Light experience just before hatching seems to trigger the lateralization (Rogers, 1982). The chick embryo is oriented in the egg such that, during the later stages of incubation, it occludes its left eye with its body while the right eye receives light input through the shell and membranes (Rogers, 1990). Young chicks have a structural asymmetry of the visual projection to the thalamo-hyperstriatum; the projections fed by the right eye to the left side of the thalamus are more numerous than those from the left eye to the right side (Rogers and Sink, 1988). This asymmetry may explain some of the lateralization in visual performances but it cannot be the only reason as the structural asymmetry is greatest in young male chicks (Adret and Rogers, 1989; Rajendra and Rogers, 1993).

While comparisons of the effect of monocular and binocular deprivation in the chick have been made (Yinon *et al.*, 1980; Sivak *et al.*, 1989), no such study comparing the effects of right and left treated eyes has been performed. While some authors routinely report which eye was treated and which served as the control, others are not consistent (Wallman *et al.*, 1978b; Yinon *et al.*, 1982/1983; Osol *et al.*, 1986), and while some treat equal numbers of right and left eyes (Lauber and Oishi, 1987) others treat all right eyes or all left (Yinon *et al.*, 1980; Hayes *et al.*, 1986). The effect of depriving the right eye compared with the left eye was investigated.

2.3.2. Methods

Animals and treatments

Male day-old White Leghorn chicks (n = 17) had one eye sutured closed (LS) for two weeks, i.e. from days 1 to 14. Due to the functional and structural asymmetry being greatest for male chicks, cockerels were used in this study. Approximately equal numbers of right (RT, n = 9) and left (LT, n = 8) eyes were deprived. Chicks were raised in temperature-controlled enclosures with food and water provided *ad libitum*. They were exposed to a 12/12 light-dark cycle, with lights on at 7 am and off at 7 pm and light intensity of 250 lux at the level of the food trough.

Measurements

Eye growth was monitored weekly for 8 weeks. Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed to determine the refractive error and the positions of the intraocular surfaces respectively. Refractive errors, anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987), under ketamine\Rhompun anaesthesia (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test was used (WSRT). To assess the difference between occlusion effects on right and left treated eyes, the Mann-Whitney U-test was used (MWUT). Dimensional changes between measurement points were used as an index of ocular growth.

2.3.3. Results

Normal ocular growth

The measured ocular parameters of normal eyes, i.e. refraction, ACD, LT, VCD, AL, and corneal power were not significantly different for the two treatment groups, i.e. right or left treated (see Appendix II, Tables AII.2.3, for normal eye data) at any age. Although not significant, the AL of normal eyes of LT birds was greater and also more variable in length than that of RT birds when the contralateral eye was opened at week 2, i.e. 9.51±0.65 mm compared with 9.16±0.32 mm. The growth rates of the anterior chamber and vitreous chamber of normal eyes were not significantly different for the two groups at any age (Fig. 2.3.5).

Form-deprivation myopia

Whether the left or right eye was form deprived, high myopia and axial elongation resulted (Table 2.3.1). Although not significant, greater myopia was observed following two weeks lid suture of the right eye, with less myopia and more variability when the left eye was sutured. During the first week of deprivation comparable changes in eye growth were recorded for both groups, with lid suture resulting in slight anterior chamber shallowing, great deepening of the vitreous chamber and associated axial elongation. The response to deprivation differed between groups during the second week, with the VCD of left-treated eyes showing no further increase relative to normal, while the interocular difference in VCD for right-treated eyes greatly increased. After 2 weeks of lid suture the mean interocular VCD difference was +1.64±0.27 mm for right-treated eyes and only +0.72±1.2 mm for left-treated eyes ($P < 0.05$, MWUT); the response of left-treated eyes was much more variable compared with the variability of right-treated eyes. While the interocular difference in axial length of left-treated eyes did increase during this period, this was due to deepening of the anterior chamber. The interocular difference in ACD of right-treated eyes was +0.43±0.25 mm and of left-treated eyes was +0.56±0.62 mm after two weeks of lid suture.

Table 2.3.1. Differences in ocular parameters, between treated eyes and normal eyes, after 1 and 2 weeks lid suture (mean ± SD; RT, n = 9; LT, n = 8).

Ocular parameter	Week 1		Week 2	
	Right T	Left T	Right T	Left T
Δ Refraction (D)	–	–	–26.3±4.8	–19.2±12
Δ Corneal power (D)	–	–	+0.98±4.2	–0.07±3.8
Δ Anterior chamber depth (mm)	–0.07±0.17	–0.08±0.21	+0.43±0.25	+0.56±0.62
Δ Axial lens thickness (mm)	+0.03±0.02	+0.02±0.02	+0.01±0.02	+0.01±0.03
Δ Vitreous chamber depth (mm)	+0.94±0.24	+0.84±0.27	+1.64±0.27*	+0.72±1.2
Δ Axial length (mm)	+0.90±0.35	+0.77±0.43	+2.09±0.32*	+1.29±1.4

*Differences between right treated and left treated groups significant at *P < 0.05, **P < 0.01, ***P < 0.05, Mann-Whitney U-test (two-tailed).*

Recovery from form-deprivation

The treated eyes of both groups showed a large hyperopic shift, i.e. decrease in myopia during the first week of normal vision; +12.84±2.6 D for right-treated eyes and +13.6±3.6 D for left-treated eyes (Fig. 2.3.5). The myopia continued to decrease during the second week with a greater decrease for right-treated eyes, i.e. +5.5±2.2 D compared with only +0.8±4.3 D for left-treated eyes. The residual myopia continued to decrease gradually and refractive errors attained normal values for both groups by week 7 (Fig. 2.3.1). Although refractions normalized the AL of treated eyes remained slightly greater than normal. Normal vision inhibited axial growth and the interocular difference in AL decreased for both groups during the first week of normal vision. This decrease continued slowly for RT chicks, however for LT chicks the AL of treated eyes grew faster than normal during the second and third weeks of recovery and remained significantly longer than normal. The interocular

difference in AL at the end of the study was 0.32±0.24 mm for right-treated eyes and 0.98±2.4 mm for left-treated eyes; the left-treated eyes also showed an extremely variable response. Decreases in axial growth were due to inhibition of growth of both the ACD and VCD.

Growth of the VCD was inhibited following eye opening and the VCD appeared to "shrink" (Fig. 2.3.5). During the first week of normal vision, the apparent "shrinkage" was 0.39±0.19 mm for left-treated eyes and only 0.02±0.27 mm for left-treated eyes; during the second week further "shrinkage" occurred for the RT group, i.e. 0.14±0.06 mm but for left-treated eyes, although growth was still much less than that for normal eyes during this period, the VCD started to increase slightly. The mean VCD of left-treated eyes were similar to normal values at 6 weeks of age. In contrast, the VCD of left-treated eyes remained slightly greater than normal for the duration of the study; this occurred even though the increase in VCD produced by lid suture was much less for the LT compared with RT group (Fig. 2.3.2).

As in earlier sections (section 2.2) growth of the anterior chamber was much slower in its recovery (Fig. 2.3.5), the anterior chamber continued to deepen faster than normal during the first week of normal vision. The increase in the ACD of right-treated eyes during this period was 0.51±0.1 mm compared with only 0.17±0.01 mm for normal eyes and increases of 0.44±0.14 mm and 0.17±0.01 mm were recorded for LT chicks respectively. Growth of the ACD was inhibited for both groups during the second week of recovery. For RT chicks the interocular ACD difference then continued to slowly decrease, but at the last measurement point the ACD of treated eyes was still greater than that of normal eyes, with an interocular difference of 0.38±0.52 mm (Fig. 2.3.2). For LT chicks, the interocular difference in ACD changed little when normal vision was restored and was still 0.78±1.6 mm at the end of the study. As for VCD the ACD response of treated left-treated eyes was extremely variable.

The lens appeared to thin during recovery compared with its normal thickness and this response was slightly greater for left-treated treated eyes (Fig. 2.3.2). Corneal steepening of treated eyes occurred during the first week of recovery; this effect was greater for the LT group (Fig. 2.3.3). The interocular differences in corneal power then gradually decreased, but for RT chicks the treated eyes corneas remained steeper than normal for the duration of the study.

Body weights

Body weights of RT and LT chicks were not significantly different at any age, although there was a tendency for RT chicks to be heavier than LT chicks (Fig. 2.3.4).

Comparison of lid suture effects to normal growth

Although AL and VCD changes were significantly greater for RT chicks, recovery for these parameters was reasonably equivalent for the two breeds. In contrast, ACD changes were only slightly greater for LT chicks at eye opening but recovery of this parameter was much poorer for LT chicks (Fig. 2.3.5). Two weeks of deprivation resulted in 22.8% increase in AL for RT treated eyes, i.e. to 11.25 mm and 13.6%, i.e. to 10.8 mm for LT chicks. The AL of normal eyes increased 36.8% and 33.1% during the 6 week recovery period for RT and LT chicks respectively; the mean AL of normal eyes were 12.53 mm and 12.66 mm at 8 weeks. Growth of the normal eye was adequate for normalization and thus fails to explain the poor normalization of AL for both groups.

The increase in AL comprised a 29.4% increase in ACD and a 30.3% increase in VCD for RT chicks; equivalent values were 36.8% and 12.7% for LT chicks. The increase in ACD continued to week 3, when percentage increases of 35.8% and 49% were observed for treated compared with normal eyes of RT and LT chicks respectively. The particularly poor normalization of ACD of LT chicks was due to the large increase in ACD of the treated eye compared with normal during the first week of normal vision. Normal anterior chamber growth for 5 weeks recovery, i.e. between weeks 3 and 8, was 34.1% and 30.2% for RT and LT chicks respectively. As for the previous sections (section 2.1 and 2.2), normal growth of the anterior chamber during the recovery period was inadequate for normalization of the deprived eye, this was true for both RT and LT groups. Normal vitreous chamber growth was 33.4% and 29.2% for RT and LT chicks during the recovery period and thus growth should have been adequate for normalization for both treatment groups. The poor normalization of the VCD of left-treated eyes is thus not explained by this model.

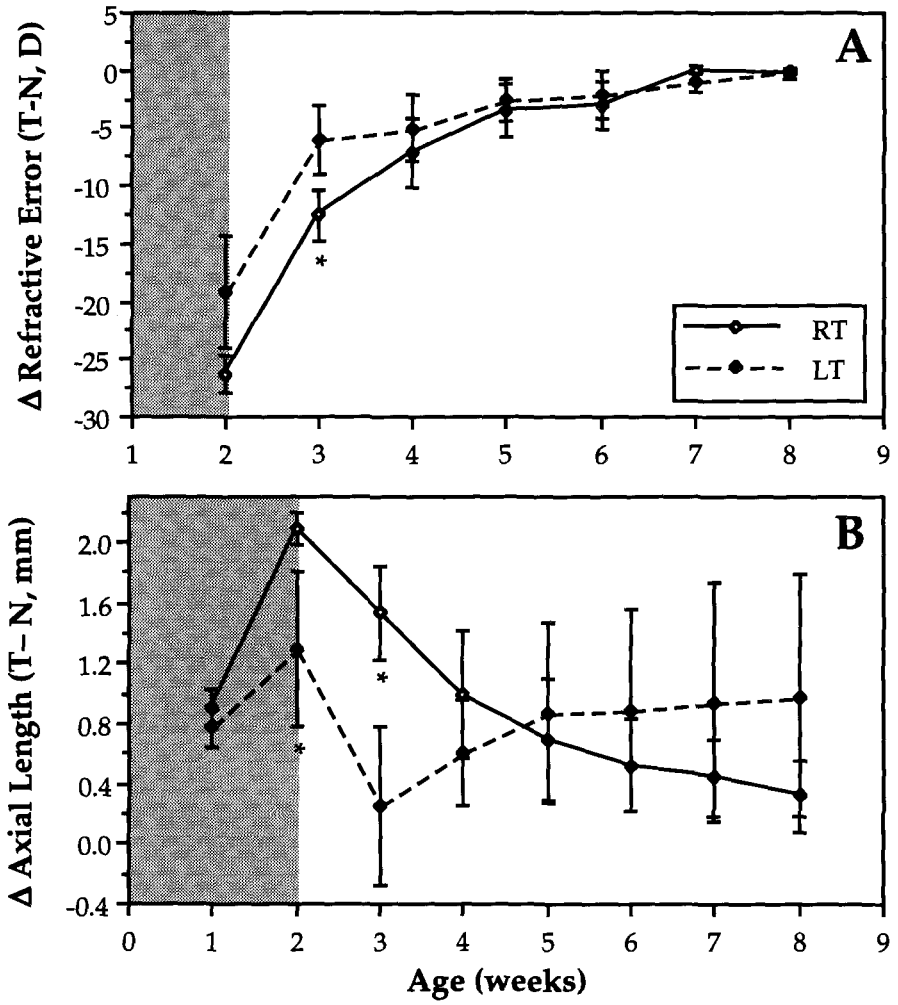


Figure 2.3.1. Differences (mean ± SE) in **A.** refraction and, **B.** axial length between treated (T) and normal (N) eyes, for 2 weeks lid suture (shaded area) and 6 weeks recovery, for RT and LT treatment groups. Differences between groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

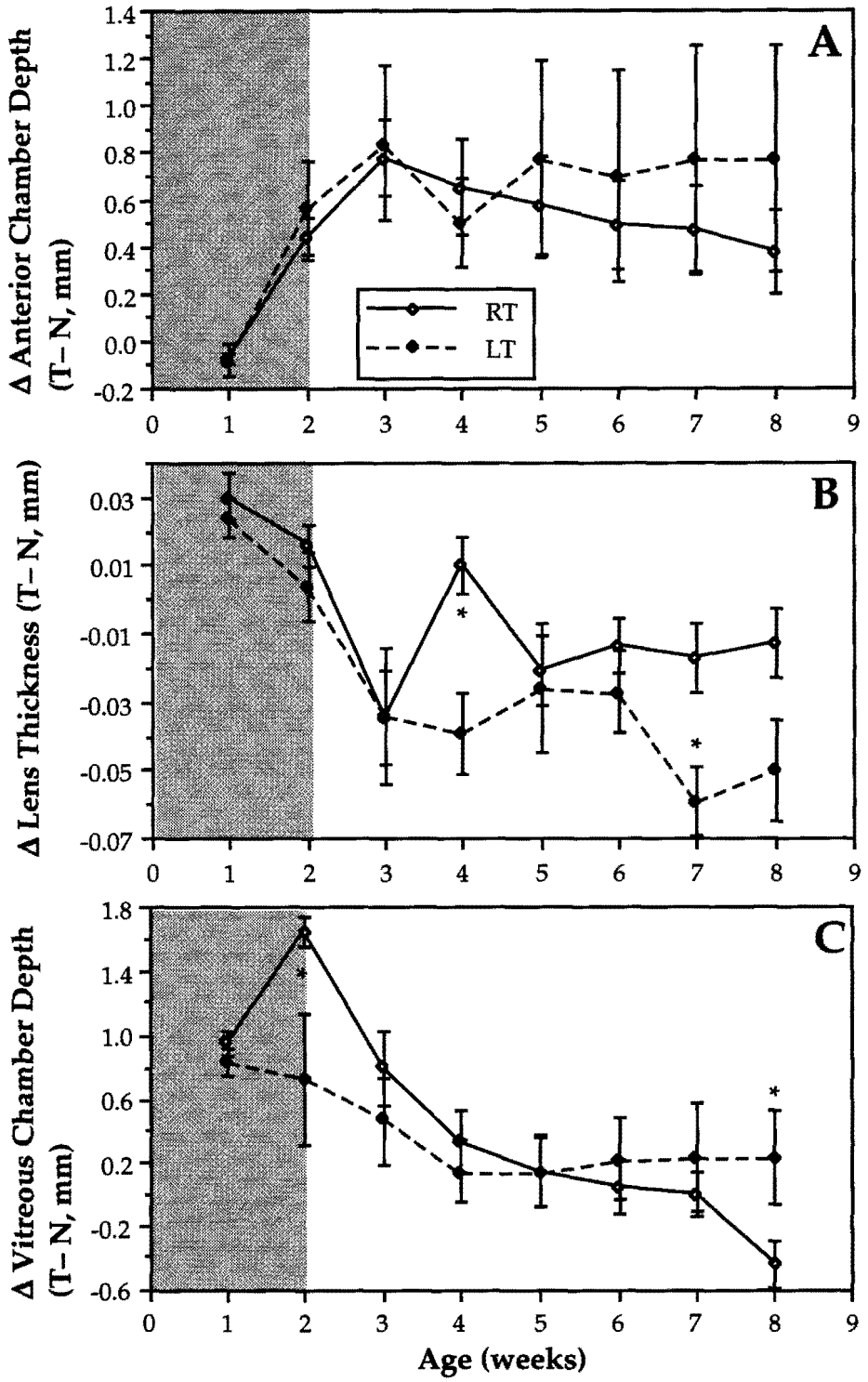


Figure 2.3.2. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** axial lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes, for 2 weeks lid suture (shaded area) and 6 weeks recovery, for RT and LT treatment groups. Differences between groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

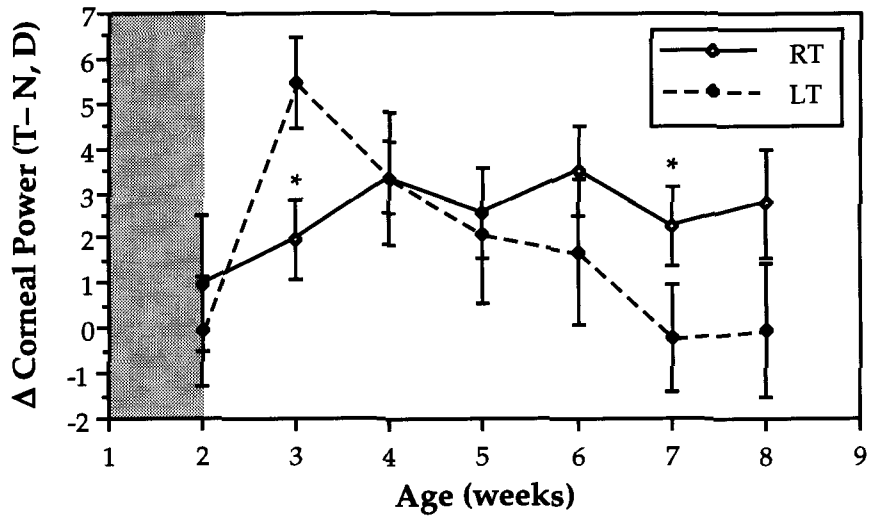


Figure 2.3.3. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes for 2 weeks lid suture (shaded area) and 6 weeks recovery, for right (RT) and left (LT) treatment groups. Differences between groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

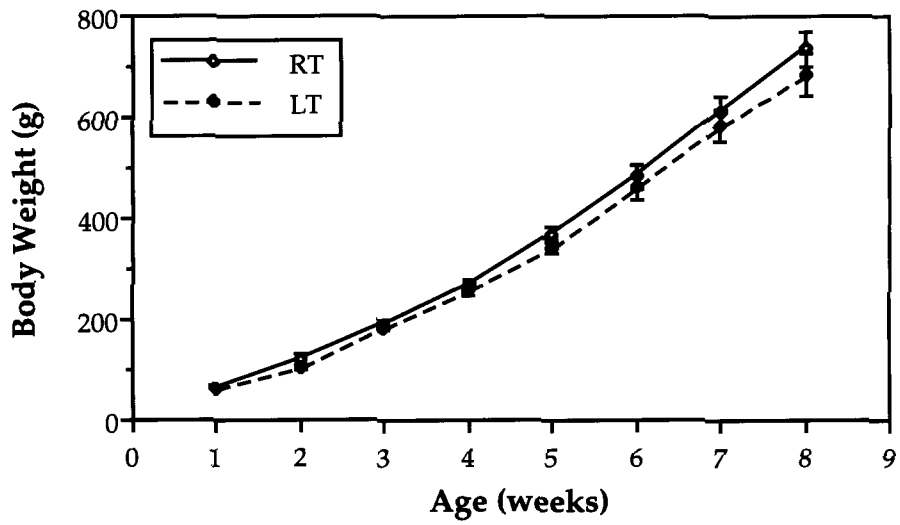


Figure 2.3.4. Body weights for RT and LT chicks (mean ± SE). Differences between groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

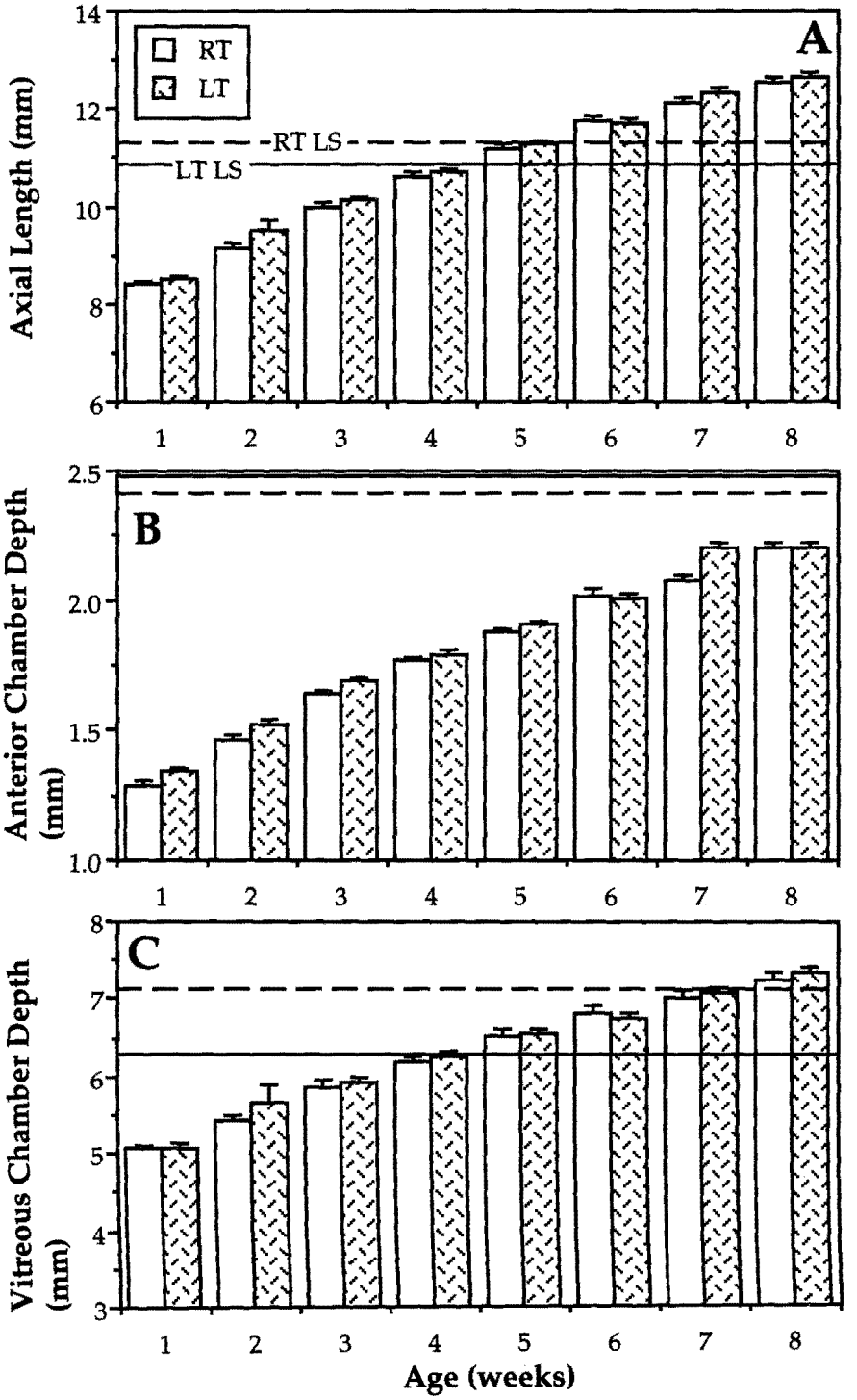


Figure 2.3.5. Comparison of lid suture response and normal growth for **A.** axial length, **B.** anterior chamber depth and **C.** vitreous chamber depth, for RT and LT treatment groups (mean ± SE). For AL and VCD the horizontal line represents the value for the treated eye following 2 weeks lid suture, for ACD the line represents the 3 week value.

2.3.4. Discussion

Normal ocular growth

There was no significant difference in the growth rates of normal eyes of the different treatment groups, i.e. right or left treated. This was the case for all the ocular parameters monitored, i.e. anterior chamber depth, lens thickness, vitreous chamber depth and axial length at all ages; refractive errors and corneal powers were also similar. This is consistent with the findings of Yinon *et al.* (1980) who reported that the ocular parameters of the open eye were not affected by depriving the fellow eye and who concluded from comparisons with untreated chicks, that the two eyes were independent with respect to the production of myopia. This was a critical finding for eye growth studies which use the non-deprived contralateral eye as a control. This result also indicates that the eyes of chicks develop largely independently of each other or that outside factors can override any developmental dependence.

Form-deprivation myopia

Whether the left or right eye was deprived, high myopia and axial elongation resulted. Although not significant, greater myopia was produced after two weeks lid suture of the right eye, with less myopia and more variability when the left was sutured. Right-treated eyes showed significantly greater vitreous chamber depth changes following 2 weeks of deprivation than did left-treated eyes, for anterior chamber depth the reverse was the case with greater anterior chamber depth changes for left-treated eyes. Although the difference between groups was not significant greater myopia may have been expected for deprivation of the right eye. The right eye of young chicks performs better on fine discrimination tasks than the left eye (Mench and Andrew, 1986) and may thus be more susceptible to image degradation. Although the above discussion for normal eyes indicated that the two eyes of the chick develop independently it has been recently suggested that form-deprivation myopia is greater when produced bilaterally (Sivak *et al.*, 1989) although this is in conflict with other earlier studies (Wallman and Adams, 1987; Schaeffel *et al.*, 1988).

Recovery from form-deprivation myopia

Recovery from form-deprivation-induced changes in ocular growth occurred for both right and left treated eyes. Although refractions returned to normal values, the axial length of treated eyes remained slightly greater than normal, with greater remaining axial elongation for left-treated eyes. Normalization of vitreous chamber depth was worst for left-treated eyes in spite of the fact that deprivation-induced increases in vitreous chamber depth were much less for the left-treated compared with right-treated group. Similarly, the anterior chamber depth of treated eyes remained greater than that of normal eyes for both groups; the residual deepening was similarly greatest for left-treated eyes.

Significance for future experiments

Due to the variability in response of left-treated eyes, equal proportions of right and left-treated eyes should be used in experiments where the results are to be compared. In experiments to follow, equal numbers of right and left treated eyes have been used when ever possible.

2.3.5. Conclusion

In conclusion, there are subtle differences in the recovery process of right and left treated eyes, thus where the effects of different treatments are to be compared equal numbers of right and left-treated eyes should be used when ever possible to exclude "eye" as a confounding variable. Alternatively, analysis including the identity of the treated eye as a variable may be a more sensitive approach, especially when the effect of a treatment on eye growth is expected to be small.

CHAPTER 3

SENSITIVITY OF THE CHICK EYE TO VISUAL DISTURBANCE

3.0. Sensitivity to Visual Disturbance

This chapter includes experiments which, while not directly investigating the nature of the visual cue to defocus, were fundamental to the correct planning of experiments to follow. The results give valuable information about the sensitivity of the chick eye to visual disturbance. Section 3.1 investigates the recovery of form-deprivation myopia following different durations of normal vision, section 3.2 compares the effect of giving periods of normal vision in the morning as opposed to in the evening, section 3.3 investigates the defocus threshold for effects on ocular growth and, section 3.4 investigates the effect of periods of normal vision on ocular compensation to spectacle-lens-induced defocus.

3.1. Sensitivity of Form-Deprivation Myopia to Normal Vision

3.1.0. Summary

The magnitude of recovery from form-deprivation myopia due to a period of normal vision was investigated. Chicks were monocularly occluded from day 1 either: i) constantly, or ii) with an interruption of 20 min, 40 min, 60 min or 120 min of normal vision at day 4. Constant occlusion produced high myopia (–12.6±3.2 D) after 5 days of treatment. When the occlusion was interrupted with a period of normal vision, some recovery occurred, mean refractions of –4.9±2.1 D, –3.8±1.6 D, –3.7±1.5 D, and –3.0±1.2 D being recorded for the 20, 40, 60 and 120 min treatment groups respectively. Although recovery was greater with increasing exposure to normal vision, only 120 min proved significantly more effective than 20 min in attenuating the response to occlusion.

3.1.1. Introduction

When young chicks are deprived of high quality form vision the emmetropization process is disturbed and excessive axial eye growth and high myopia results (Wallman *et al.*, 1978b). Once normal vision is restored refractive recovery from the myopia occurs extremely rapidly (Wallman and Adams, 1987); recovery from 20 D of myopia can occur in less than a week (section 2). Presumably a myopic defocus signal is detected once normal vision is restored resulting in compensatory eye growth. An unanswered question is: what is the duration of normal vision required for detection of the defocus signal?

The anomalous ocular growth resulting from deprivation can be inhibited by interruption of the form-deprivation treatment with periods of normal visual stimulation; as little as 2 hrs per day totally prevents form-deprivation myopia (Nickla *et al.*, 1989). This would indicate that less than 2 hrs of normal vision are required to maintain an emmetropic refraction.

The ability of one short period of normal vision to drive recovery from occlusion-induced myopia was investigated. It was expected that if the duration of normal vision was increased in a stepwise manner, then the ability of this stimulus to reduce occlusion-induced ocular changes would likewise increase in a stepwise fashion and that there would be some critical period below which recovery would not be observed. This study also has relevance to experiments that follow which used an interrupted occlusion paradigm.

3.1.2. Methods

Animals and treatments

Male White Leghorn-New Hampshire crossbreed chicks were obtained from a local hatchery on the day of hatching. They were raised in temperature controlled enclosures with food and water provided *ad libitum*. Chicks were exposed to a 12 hr/12 hr light-dark cycle, with lights on at 7 am and off at 7 pm, and a light intensity of 250 lux at the level of the food trough. Two groups of 15 chicks were used in this study with a total of 6 chicks being assigned to each of 5 different experimental conditions.

Chicks were monocularly occluded from day 1 and either: i) remained constantly occluded (CO or 0), or ii) had occlusion interrupted with a 20 min (20), 40 min (40), 60 min (60) or 120 min (120) period of normal vision on day 4 post hatching. Ocular parameters were measured on day 5, i.e. approximately 24 hrs after the period of normal vision.

Measurements

Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed under dim illumination to determine the refractive error and the positions of the intraocular surfaces respectively. Anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. An estimate of combined retinal and choroidal thickness was made using A-scan ultrasound. Corneal curvature was measured by infrared photokeratometry (Schaeffel and Howland, 1987), under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test (WSRT) was used. To assess the effect of varying the duration of normal vision the interocular differences between eyes of different treatment groups was compared using the Mann-Whitney U-test (MWUT; see Appendix I for more details). In the results section data are reported as mean ± SD unless otherwise indicated.

3.1.3. Results

Constant occlusion

Constant monocular deprivation resulted in high myopia; at day 5 the deprived eyes were an average −12.6±3.2 D ($P < 0.005$, WSRT) more myopic than contralateral normal eyes (Table 3.1.1; Fig. 3.1.1). The myopia was primarily due to increased growth of the vitreous chamber, 0.46±0.09 mm increase relative to normal eyes ($P < 0.005$, WSRT; Fig. 3.1.2). There was also an associated increase of 0.50±0.12 mm ($P < 0.005$, WSRT; Fig. 3.1.1) in AL for treated eyes. Slight deepening of the anterior chamber, by

0.04±0.04 mm ($P < 0.05$, WSRT, Fig. 3.1.2), and corneal steepening, by 2.5±2.3 D ($P < 0.05$, WSRT; Fig 3.1.3) were observed. There was no effect of constant occlusion on measured lens thickness (see Appendix II, Table AII.3.1, for treated and normal eye data).

Effect of introducing one period of normal vision

Introducing one period of normal vision on day 4 had a marked effect on the occlusion-induced changes (Table 3.1.1). All periods of normal vision tested were equally effective at reducing form-deprivation myopia. The magnitude of form-deprivation myopia was –4.9±2.1 D (39% of CO levels, $P < 0.005$, MWUT; Fig. 3.1.1), –3.8±1.6 D (30% of CO levels, $P < 0.005$, MWUT), –3.7±1.5 D (29% of CO level, $P < 0.005$, MWUT), and –3.0±1.2 D (24% CO of level, $P < 0.005$, MWUT) for the 20, 40, 60 and 120 min treatment groups respectively. These values were compared with the equivalent value of –12.6±3.2 D for the CO group, the statistical data in brackets relates to comparisons with this group. Although slightly greater reductions in myopia were observed with longer periods of visual stimulation, the correlation between degree of myopia prevented and duration of normal vision was not significant at $P < 0.05$ (Fig 3.1.4).

Occlusion-induced vitreous chamber elongation was markedly decreased when the occlusion was interrupted with normal vision. Vitreous chamber elongation of 0.29±0.09 mm (63% of CO level, $P < 0.005$, MWUT; Fig 3.1.2), 0.25±0.10 mm (54% of CO level, $P<0.005$, MWUT), 0.24±0.07 mm (52% of CO level, $P < 0.005$, MWUT), and 0.16±0.08 mm (35% CO of level, $P < 0.005$, MWUT) occurred for the 20 min, 40 min, 60 min and 120 min treatment groups respectively. Again the statistics relate to comparisons between these and the CO group. The 120 min period of normal vision was statistically more effective than 20 min at reducing the effect of occlusion on the vitreous chamber, and a similar statistically significant difference in AL effects were also recorded. The 120 min group also differed significantly from both the 40 min and 60 min groups with respect to both of these parameters. There was no significant difference in the treatment effects on vitreous chamber and axial elongation for any of the other normal visual stimulation groups. The correlation between axial elongation and duration of visual stimulation was only significant if the zero, i.e. CO, data point was excluded ($r = 0.979$, $P < 0.05$; Fig. 3.1.4).

Table 3.1.1. Differences in ocular parameters, on day 5, between treated and normal eyes of constant occlusion group and interrupted occlusion groups (20 min, 40 min, 60 min, 120 min; mean ± SD, n = 6 for all groups).

Ocular parameter	CO/ 0	20	40	60	120
Δ Refraction (D)	–12.6±3.2	–4.9±2.1***	–3.8±1.6***	–3.7±1.5***	–3.0±1.2***
Δ Corneal power (D)	+2.5±2.3	–0.6±3.9*	+0.6±3.7	–0.7±1.5*	+0.04±5.1
Δ Anterior chamber depth (mm)	+0.04±0.04	+0.002±0.04**	–0.008±0.01**	+0.01±0.03*	+0.00±0.04*
Δ Axial lens thickness (mm)	0.00±0.01	–0.01±0.01	+0.02±0.01	–0.002±0.01	–0.007±0.01
Δ Vitreous chamber depth (mm)	+0.46±0.09	+0.29±0.09***••	+0.25±0.10***•	+0.24±0.07***•	+0.16±0.08***
Δ Axial length (mm)	+0.50±0.12	+0.29±0.09***••	+0.24±0.08***•	+0.24±0.07***•	+0.15±0.08***

*Differences between constant occlusion and interrupted occlusion groups significant at *P < 0.05, **P < 0.01, ***P < 0.005. Differences between the 120 min group and other interrupted occlusion treatment groups (20, 40, 60) significant at •P < 0.05, ••P < 0.01, •••P < 0.005; Mann-Whitney U-test (one-tailed).*

All interrupted occlusion treatment groups showed negligible deepening of the anterior chamber; this contrasts with anterior chamber deepening that was observed in the constant occlusion group. Equivalent values were 0.04±0.04 mm for the CO group compared with only 0.002±0.04 mm ($P < 0.01$, MWUT), 0.008±0.01 mm ($P < 0.01$, MWUT), 0.01±0.03 mm ($P < 0.05$, MWUT), and 0.00±0.04 mm ($P < 0.05$, MWUT) for the 20 min, 40 min, 60 min and 120 min treatment groups respectively. All differences relative to the CO data were statistically significant as indicated. Interruption of occlusion also decreased the corneal steepening seen with constant occlusion. In some cases the reverse effect, i.e. slight corneal flattening, was observed. Corneal power changes of –0.6±3.9 D ($P < 0.05$, MWUT), –0.6±3.7 D, +0.7±1.5 D ($P < 0.05$, MWUT), and +0.04±5.1 D were recorded for the 20 min, 40 min, 60 min and 120 min treatment groups

respectively. Axial lens thickness was unaffected by occlusion and there was no measured effect on lens thickness of interrupted occlusion.

Values of 0.42±0.07 mm, 0.42±0.01 mm, 0.46±0.08 mm, and 0.52±0.07 mm were obtained for combined retina and choroidal thickness for treated eyes of the 20 min, 40 min, 60 min, and 120 min treatment groups respectively. Measurements on normal eyes and constantly occluded eyes were not possible due to the combined retina and choroidal thickness being below the resolution limit of the available A-scan system.

Predicted changes in refraction based on ocular parameter changes

Predictions of changes in refraction based on measured changes in ACD and VCD were similar to those measured using retinoscopy for all treatment groups (Table 3.1.2). This result confirmed the axial nature of both the myopia observed and the significant contribution of VCD changes to refractive changes.

Table 3.1.2. Predicted (based on ocular parameter changes) compared with measured changes in refraction for constant occlusion (CO) and interrupted occlusion (20 min, 40 min, 60 min and 120 min) treatment groups at day 5 (mean ± SD, n = 6 for all groups).

	CO/ 0	20	40	60	120
Measured Δ RE (D)	–12.6±3.2	–4.9±2.1	–3.8±1.6	–3.7±1.5	–3.0±1.2
Δ RE ACD (D)	–1.2	–0.06	+0.23	–0.29	0
Δ RE VCD (D)	–7.3	–4.6	–4.0	–3.8	–2.5
Measured Δ CP (D)	+2.5±2.3	–0.6±3.9	+0.6±3.7	–0.7±1.5	+0.04±5.1
Predicted Δ RE (D)	–11.0	–4.1	–4.4	–3.4	–2.5

Predicted ΔRE based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

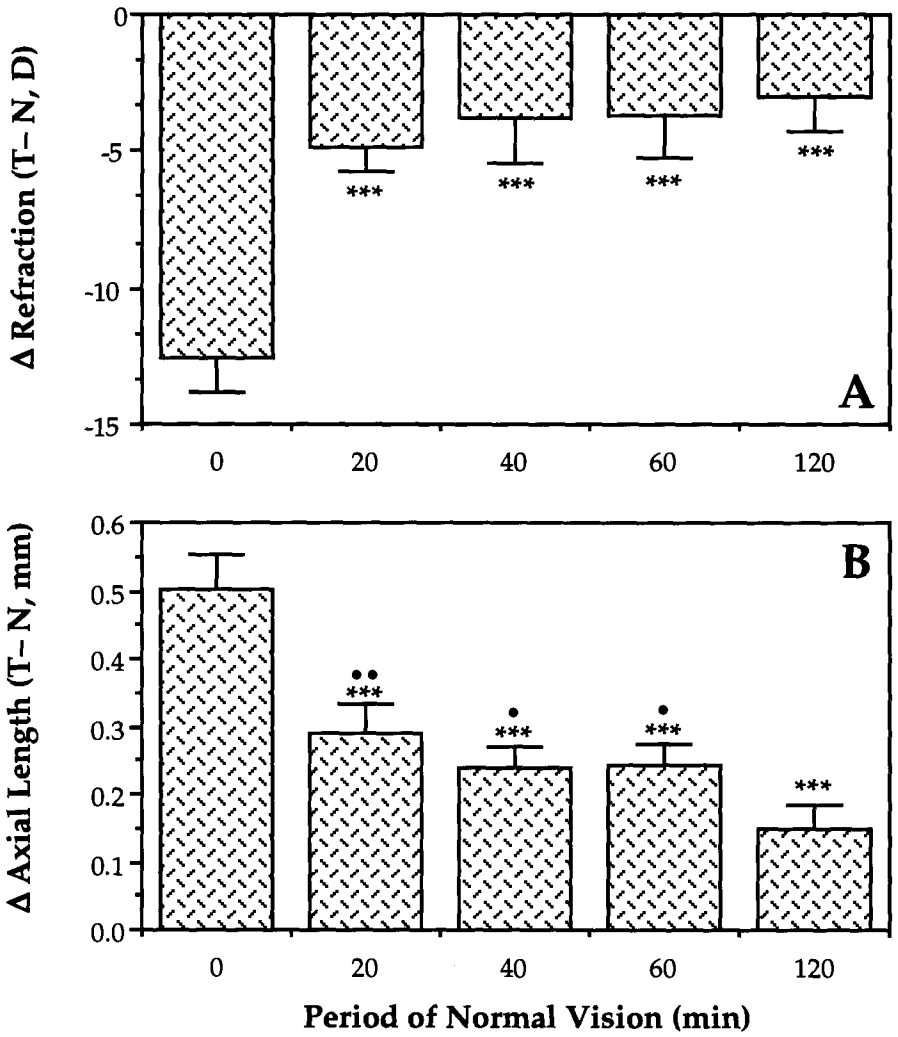


Figure 3.1.1. Differences (mean ± SE) in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes, at day 5, after constant occlusion (0) or occlusion interrupted with one period of normal vision (20, 40, 60, 120 min) on day 4. Differences between constant occlusion and interrupted occlusion groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between 120 min and other interrupted occlusion treatment groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

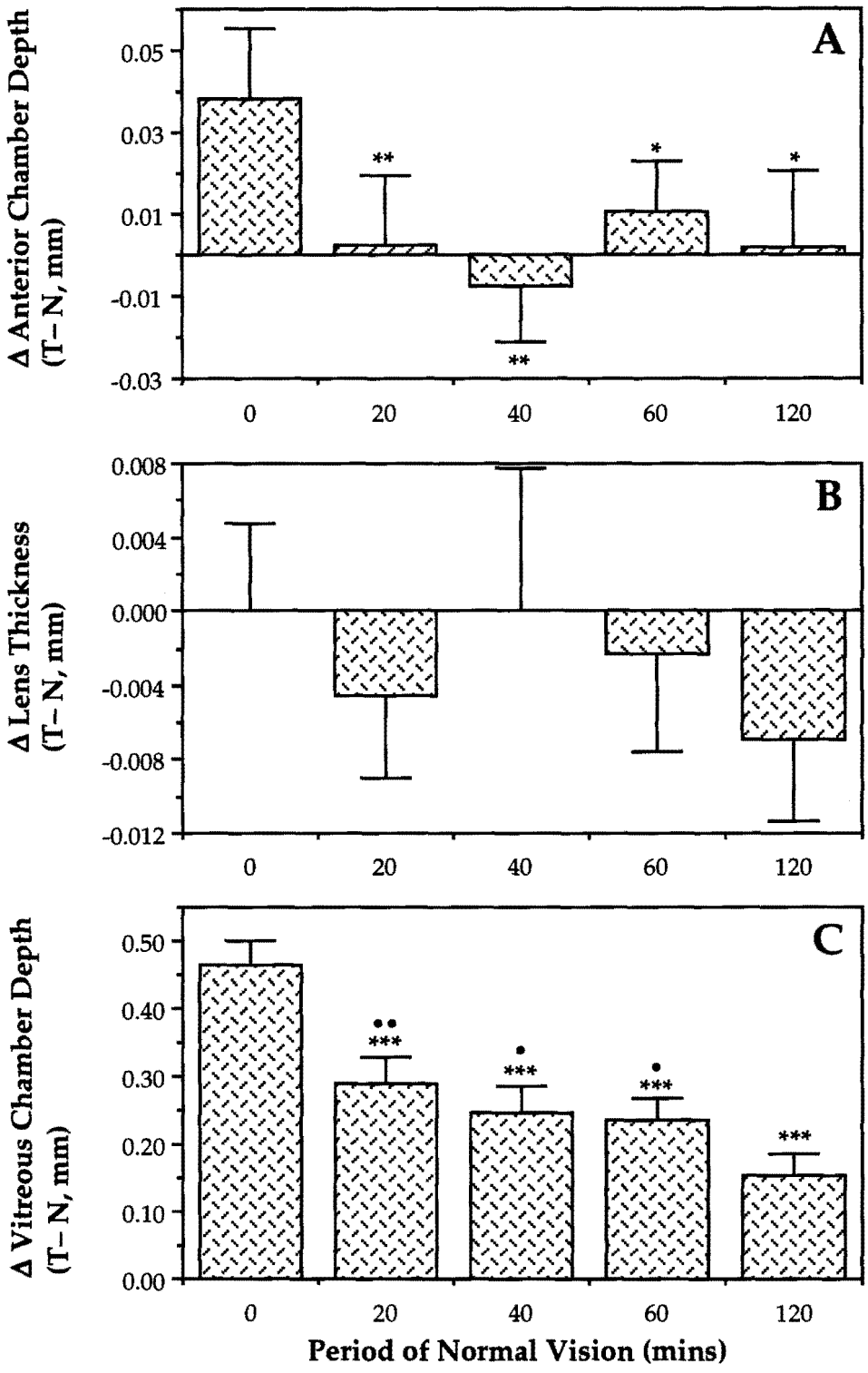


Figure 3.1.2. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes, at day 5, after constant occlusion (0) or with occlusion interrupted on day 4 (20, 40, 60, 120 min). Differences between constant occlusion and interrupted occlusion groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between 120 min and other interrupted occlusion treatment groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

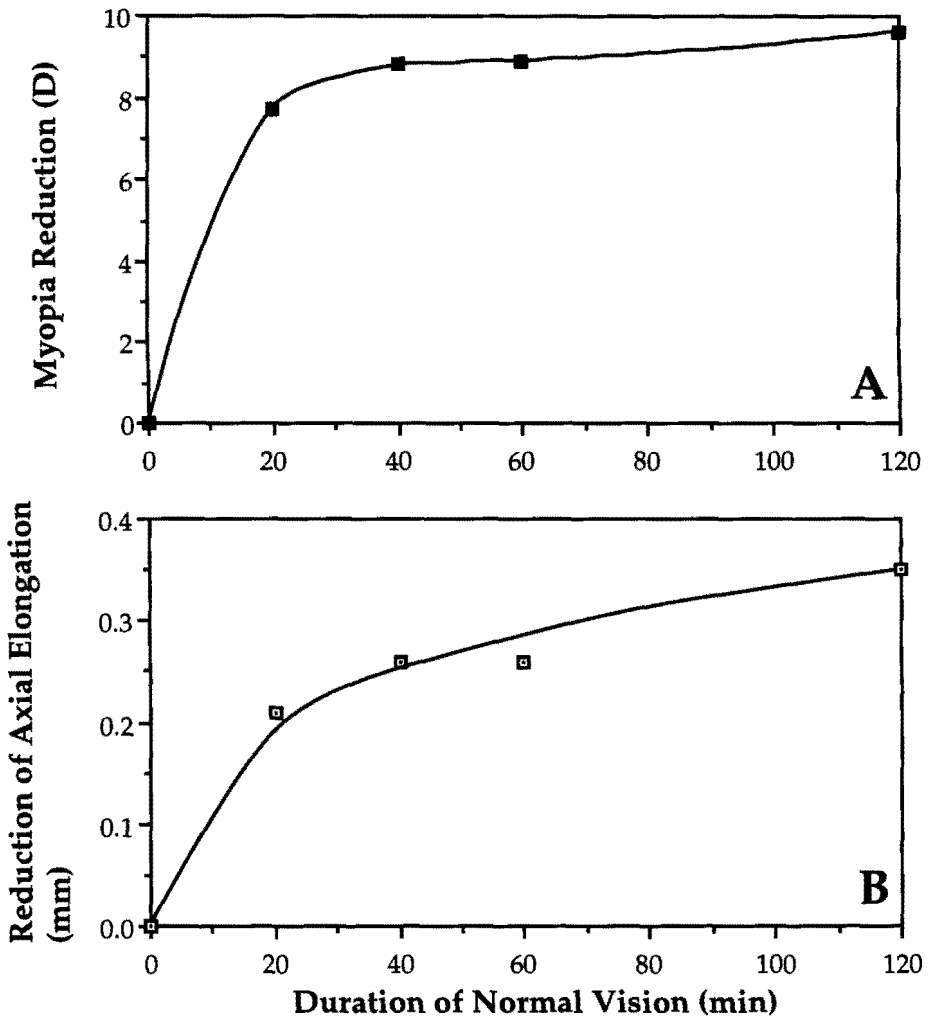


Figure 3.1.4. Magnitude of **A.** myopia and **B.** axial elongation reduction (reduction = Δ 0 – {Δ 0, Δ 20, Δ 40, Δ 60, Δ 120 min}) compared with the duration of the period of normal visual stimulation. Although the amount of myopia reduction increased with the duration of visual stimulation the correlation between refractive error and time was not significant at $P < 0.05$. The correlation between reduced axial elongation and duration of visual stimulation was only significant ($r = 0.979$, $P < 0.05$) when the zero (CO) data point was excluded.

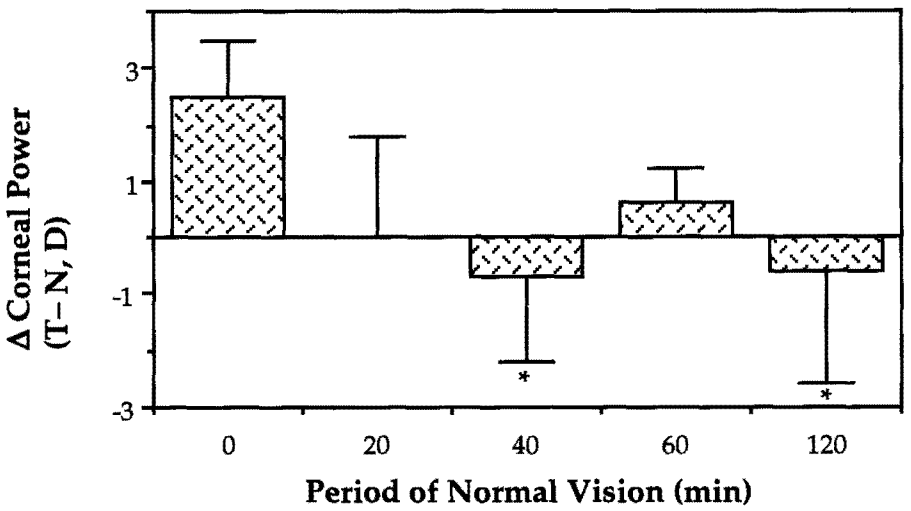


Figure 3.1.3. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes, at day 5, after constant occlusion (0) or with occlusion interrupted on day 4 (20, 40, 60, 120 min). Differences between constant occlusion and interrupted occlusion groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

3.1.4. Discussion

Duration of visual stimulation

A single period of normal vision on day 4 was highly effective in reducing occlusion-induced changes in eye growth, i.e. decreasing the magnitude of myopia and axial expansion measured on day 5. Periods of normal vision from 20 min to 60 min were equally effective. It was not until the period of normal vision was increased to 120 min, that a significant further reduction in the occlusion effect on refractive error and AL was observed. Expansion of the vitreous chamber contributed most to the measured refractive error for all treatment groups.

It was predicted that, as the duration of normal vision was increased that the reduction of both myopia and axial elongation would increase in a stepwise fashion; this did not occur. Increasing the duration of normal vision from 20 min to 60 min did not result in 3 times the myopia prevention. Similarly, increasing the period of stimulation from 20 min to 120 min, resulted in only twice rather than 6 times the magnitude of myopia prevention.

Prolonged effect of normal vision on ocular growth

As the degree of myopia reduction was not significantly correlated to the duration of the normal vision it seems that the period of normal vision continued to have an effect, i.e. to drive the refraction towards emmetropia even when the occluder had been replaced. It is also interesting to speculate that, during form deprivation, some retinal growth inhibitory substance accumulates and when normal vision is experienced, even as a brief "pulse", this substance is released perhaps also as a short lived pulse. As all treatment groups experienced equivalent periods of deprivation before the period of normal vision, this could explain their similarity of effect.

An alternative possibility is that the defocus state of the eye was sampled immediately on removal of the occluders, setting in motion an emmetropizing response. If the response is relatively slow, it would be of no benefit to continue sampling as the "same" defocus errors will still be present. The increased effectiveness of the 120 min group could indicate that the image was sampled twice in this period or could reflect the decrease in the duration of the subsequent occlusion. The continued accelerated growth of the constantly occluded group is also likely to contribute to the large recovery effect of one period of normal vision in young chicks.

This experiment is in fact a "mirror image" of that reported by Nickla *et al.* (1989) who showed that form-deprivation myopia was prevented by as little as two hours of normal vision per day. Unlike their study where refractions started at emmetropia, in the study reported here quite large myopic refractive errors were present before the period of normal vision was experienced. It is interesting to speculate that the same signals underlie both effects, perhaps simply varying in amplitude depending on the nature of the "pre-existing" growth processes that must be altered.

Choroidal expansion

Combined retinal and choroidal thickness values were obtained for the treated eyes of interrupted occlusion groups only. Choroidal thickness has been reported to be approximately 0.23 mm in normal chick eyes and is usually thinner than normal for constantly deprived eyes (Wallman *et al.*, 1992). Extrapolation to thickness data reported here indicates choroids

of treated eyes for all interrupted occlusion groups were expanded approximately two fold. Choroidal expansion has been put forward in explanation for the rapid recovery from form-deprivation myopia that is seen in young chicks (Wallman *et al.*, 1992). These estimates of choroidal expansion account entirely for the difference in expansion of vitreous chamber between the constant occlusion groups and other "interrupted occlusion" groups. Estimated changes in choroidal thickness of approximately 0.19 mm (20 min group) to 0.29 mm (120 min group) would account for 3.0 D, i.e. 40%, to 4.5 D, i.e. 50%, of the observed reductions in myopia. The decrease in anterior chamber effect with interrupted occlusion, would explain some of the changes in myopia not accounted for by choroidal changes.

Significance for future experiments

The results show that even a very brief period of normal vision on day 4, can greatly decrease occlusion-induced myopia and axial expansion measured on day 5. This result has extreme relevance to experiments that follow which use interrupted occlusion paradigms. Thus if an occluder inadvertently fell off, even for a very short duration, the data from that experimental animal was rejected. While Nickla *et al.* (1989) reported that a longer period of normal vision, i.e. 2hrs, was required to prevent the development of form-deprivation myopia in their chicks, the effect of shorter periods was not reported.

3.1.5. Conclusion

Interrupting occlusion with even a very short period of normal vision can have a marked effect on ocular growth. Periods of normal vision from 20 min to 60 min were equally effective at reducing the high myopia and increased axial eye growth seen with constant visual deprivation in young chicks; it was not until the period of normal vision was increased to 120 min that a further reduction in both myopia and axial expansion was observed.

3.2. The Effect of Timing of Normal Vision on the Prevention of Form-Deprivation Myopia in Chicks.

3.2.0. Summary

Chicks were monocularly occluded from hatching and either constantly occluded or given 20 min of normal vision, at either: i) the start, or ii) the end of the light cycle. After 10 days of constant occlusion –20.9±7.3 D of myopia was produced. Periods of normal vision prevented a large amount of the myopia, with –5.8±3.2 D and –5.4±2.2 D of residual myopia for visual stimulation instigated in the morning and afternoon, respectively. Thus the timing of normal vision does not affect the extent to which form-deprivation myopia is decreased by the experience. The data did not support the prediction that normal vision would be more effective at preventing form-deprivation myopia if given in the afternoon compared with in the morning.

3.2.1. Introduction

It has been recently shown that there is a diurnal cycle of ocular growth in the chick; chick eyes appear to increase their axial length only during the day, with growth being inhibited during the night (Weiss and Schaeffel, 1993). It was also shown that in occluded eyes the diurnal rhythm in eye growth was lost; while daytime growth was not affected, occlusion prevented the inhibition of growth at night. This result seems contrary to expectations given that the effect of occluders is to interfere with vision, which can only be experienced in the light and thus during the day.

Form-deprivation myopia is extremely sensitive to brief periods of normal vision, at least for young chicks, with as little as 2 hrs of normal vision per day totally preventing form-deprivation myopia (Nickla *et al.*, 1989) and periods of normal vision as short as 20 min greatly reducing the effect of occlusion (section 3.1). As the exaggerated eye growth that causes myopia appears to occur during the night (Weiss and Schaeffel, 1993) it was speculated that the timing of the period of normal vision, i.e. morning compared with afternoon, would influence the sensitivity of the form deprivation response to this interruption. An experiment was designed to examine this possibility. It was predicted that periods of normal vision in the afternoon would be more effective at preventing

occlusion-induced myopia than periods of normal vision in the morning, due to the difference in proximity to the period when the anomalous growth that causes myopia occurs. Afternoon exposure to normal vision might serve to switch the eye back to a "normal growth mode" prior to darkness. This pattern of growth may then be maintained for the entire dark period. In the case of normal vision in the morning, the "normal growth mode" is assumed to be switched off by subsequent form-deprivation and thus excessive axial growth occurs for most of the lighted and dark hours. This hypothesis was tested by giving occluded chicks periods of normal vision either in the morning, i.e. at the beginning of the light cycle, or in the afternoon, i.e. at the end of the light cycle. This study also has relevance to experiments that follow which use interrupted occlusion or interrupted lens wear paradigms.

3.2.2. Methods

Animals and treatments

Male White Leghorn-New Hampshire crossbreed chicks were obtained from a local hatchery on the day of hatching. They were raised in temperature controlled enclosures with food and water provided *ad libitum*. Chicks were exposed to a 12 hr/12 hr light-dark cycle, with lights on at 7 am and off at 7 pm and a light intensity of 250 lux at the level of the food trough. Two groups of 12 chicks were used in this study with a total of 8 chicks being assigned to each of three different experimental conditions.

Chicks were monocularly occluded from day 2 and either: i) constantly occluded (CO), ii) given 20 minutes of normal vision in the morning, i.e. at the start of the light cycle from 7.30 am to 7.50 am (am), or iii) given 20 minutes of normal vision in the afternoon, i.e. at the end of the light cycle from 6.10 pm to 6.30 pm (pm).

Measurements

Ocular parameters were measured on day 10, following 9 days of treatment. Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed in a dim room to determine the refractive error and the positions of the intraocular surfaces respectively. Anterior chamber depth (ACD), axial

lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987), under ketamine/Rhompun anaesthesia.

Chicks were finally sacrificed using sodium pentobarbitone. The eyes were enucleated, cleared of extraneous muscle tissue and the axial length and equatorial diameters measured directly with digital calipers. Eyes were also weighed on an electronic balance (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and control eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test (WSRT) was used. To assess the difference between morning (am) and afternoon (pm) treatment groups, the Mann-Whitney U-test (MWUT) was used to compare interocular differences (see Appendix I for more details).

3.2.3. Results

After 10 days of constant occlusion, occluded eyes were highly myopic; being on average –20.9±7.3 D ($P < 0.005$, WSRT) more myopic than the associated normal eyes (Table 3.2.1; Fig 3.2.1). This myopia was partially prevented by short daily periods of normal vision. Periods of normal vision in the morning were as effective as periods in the afternoon at preventing form-deprivation myopia. Refractive changes of –5.8±3.2 D and –5.4±2.2 D were recorded for the am and pm treatment groups respectively (see Appendix II, Tables AII.3.2, for treated and normal eye data).

After 10 days of constant occlusion, treated eyes showed significant expansion of the vitreous chamber (Fig. 3.2.2; $P < 0.005$, WRST); occluded eyes showed an average increase of 0.76±0.32 mm in vitreous chamber growth compared with normal eyes. Periods of normal vision in the morning were as effective as periods in the afternoon at preventing occlusion-induced increases in VCD. With 20 min of normal vision increases in VCD of 0.34±0.19 mm and 0.32±0.17 mm were recorded for the am and pm treatment groups respectively; these values represent reductions of approximately 55% compared with changes in VCD in the

CO groups. There was no statistical difference between the two intermittent occlusion groups with respect to changes in VCD.

For all three groups, changes in AL reflected those for VCD (Fig. 3.2.1), with vitreous chamber elongation contributing 85%, 80%, and 71% of the axial elongation for the CO, am and pm treatment groups respectively. Constant occlusion resulted in an average increase of 0.90±0.32 mm increase in axial eye growth, compared with increases of 0.43±0.17 mm and 0.45±0.19 mm for the am and pm treatment groups, respectively. The changes in AL were highly correlated with changes in VCD (Fig. 3.2.3); correlation was better for the constant occlusion group (r = 0.961, $P < 0.001$) and poorer for the interrupted occlusion groups (r = 0.775, $P < 0.05$, am; r = 0.690, $P < 0.05$, pm).

Constant visual deprivation resulted in deepening of the anterior chamber by 0.12±0.13 mm (Fig. 3.2.2; $P < 0.005$, WSRT); this accounted for approximately 13% of the observed changes in AL. Periods of normal vision were not very effective at preventing occlusion-induced changes in the anterior chamber. For the am treatment group, a mean difference of 0.10±0.14 mm between ACDs of treated and normal eyes was recorded; this represent a 23% contribution to the measured axial elongation. For the pm treatment group, the changes in the anterior chamber were greater, although not significantly so, than those of the constant occlusion group, with an average increase of 0.16±0.14 mm in ACD representing a 35% contribution to the measured axial elongation for this group. The greater contribution of the anterior chamber to the measured axial elongation for the interrupted treatments groups was also reflected in the weak correlation between induced changes in VCD and AL.

Both intermittent occlusion groups showed less corneal steepening than the full-time occlusion group, for which a mean increase in corneal power of 1.3±3.9 D was observed (not significant; Fig. 3.2 4). Slight, but not significant, corneal flattening of 0.53±3.4 D and 0.3±3.1 D was recorded for the am and pm treatment groups respectively.

The trends in ultrasound data were reinforced with measurements of enucleated eyes. Constant occlusion resulted in increased external axial length, equatorial diameter (AL and EQD, Fig. 3.2.4) and eye weight (Fig. 3.2.6) compared with contralateral normal eyes (AL, EQD and weight, $P < 0.005$, WSRT). These increases were significantly less when brief periods of normal vision were introduced (AL, EQD and weight, $P < 0.005$, MWUT, am and pm). Normal vision in the morning as opposed to the afternoon was most effective in preventing occlusion-induced increases

in external axial eye growth ($P < 0.01$, MWUT) and equatorial diameter ($P < 0.01$, MWUT). The differences between am and pm groups were significant at $P < 0.01$ (MWUT) for both external axial length and equatorial diameter. Although the change in eye weight was slightly less with the am compared with pm treatment groups this difference was not significant.

Table 3.2.1. Interocular differences for constant occlusion (CO), and intermittent occlusion, (am) and (pm), treatment groups at day 10 (mean ± SD, n = 8 for all groups).

Ocular parameter	CO	am	pm
Δ Refraction (D)	−20.9±7.3	−5.8±3.2***	−5.4±2.2***
Δ Corneal power (D)	+1.3±3.9	−0.5±3.4	−0.3±3.1
Δ Anterior chamber depth (mm)	+0.12±0.13	+0.10±0.14	+0.16±0.14
Δ Axial lens thickness (mm)	+0.01±0.06	−0.01±0.03	−0.03±0.02
Δ Vitreous chamber depth (mm)	+0.76±0.32	+0.34±0.19***	+0.32±0.17***
Δ Axial length (mm)	+0.90±0.32	+0.43±0.17***	+0.45±0.19***

*Differences between constant occlusion and normal vision groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed). There was no significant difference between the am and pm treatment groups for any of the ocular parameters studied.*

Predictions of changes in refraction based on measured changes in the ocular parameters and the schematic eye of Schaeffel and Howland (1988) were made. Predictions slightly underestimated the myopia produced by constant occlusion and slightly overestimated the magnitude of myopia for the interrupted occlusion treatment groups. The analysis also highlighted the much greater contribution of the ACD changes to the predicted myopia for the interrupted occlusion treatment groups compared with constant occlusion (Table 3.2.2).

Table 3.2.2. Predicted compared with measured changes in refractive error (RE) for constant occlusion (CO), and intermittent occlusion, (am) and (pm), treatment groups at day 10 (mean ± SD, n = 8 for all groups).

	CO	am	pm
Measured Δ RE (D)	−20.9±7.3	−5.8±3.2	−5.4±2.2
Δ RE ACD (D)	−3.5	−2.9	−4.6
Δ RE VCD (D)	−12.0	−5.4	−5.0
Measured Δ CC (D)	+1.3±3.9	−0.5±3.4	−0.3±3.1
Predicted Δ RE (D)	−16.8	−7.8	−9.3

Predicted ΔRE based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

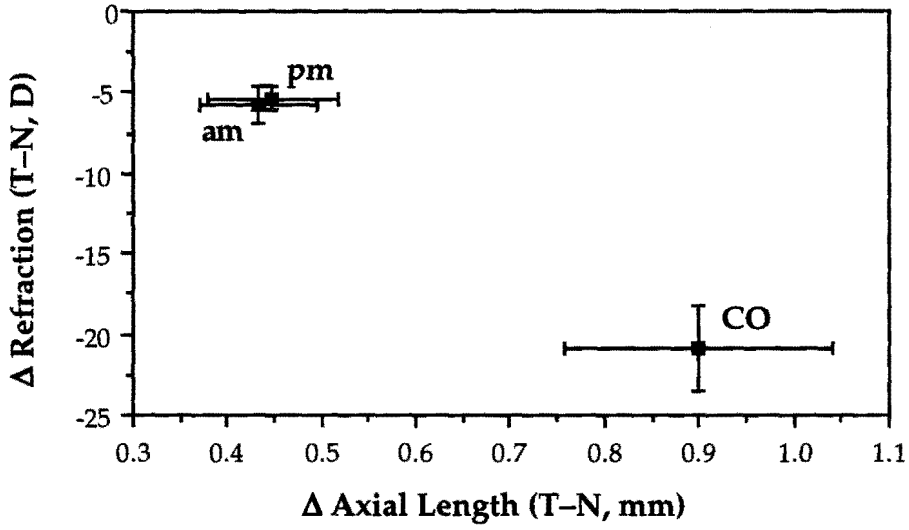


Figure 3.2.1. Relationship between differences in axial length and refraction between treated (T) and normal (N) eyes, at day 10, for constant occlusion (CO), and interrupted occlusion groups (am, pm; mean ± SE). Periods of normal vision in the morning and afternoon were equally effective at preventing form-deprivation myopia and axial elongation.

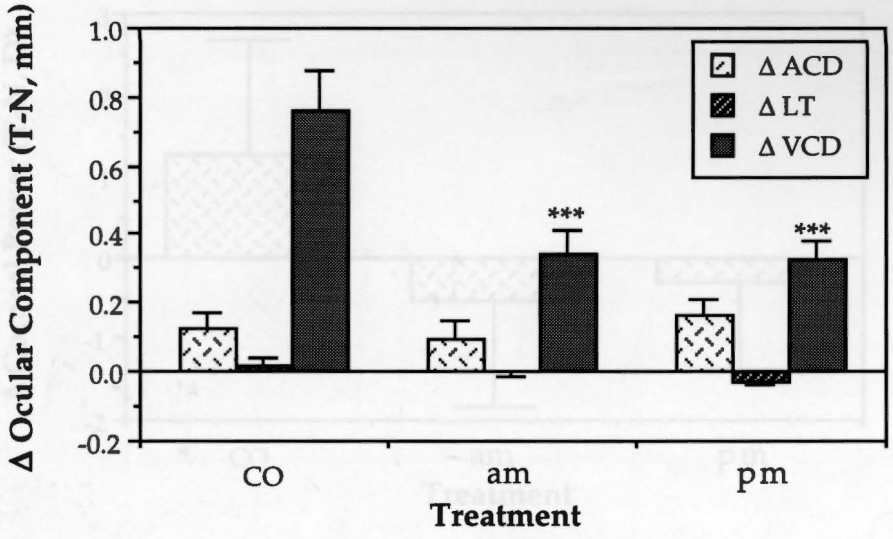


Figure 3.2.2. Differences at day 10, in anterior chamber depth (ACD), lens thickness (LT) and vitreous chamber depth (VCD) between treated (T) and normal (N) eyes, for constant occlusion (CO) and interrupted occlusion groups (am, pm; mean ± SE). Differences between CO and interrupted occlusion treatment groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed). There was no significant difference between the am and pm treatment groups for any of the ocular parameters studied.

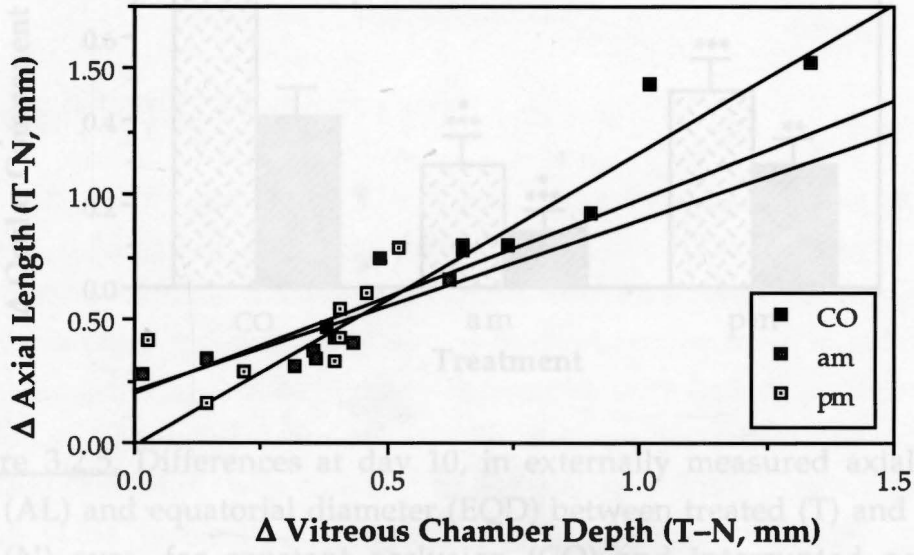


Figure 3.2.3. Correlation between differences in vitreous chamber depths (VCD) and axial lengths (AL) between treated (T) and normal (N) eyes for CO, am and pm treatment groups. The correlation was significant for all groups, P < 0.001, P < 0.05, and P < 0.05 for the CO, am and pm groups respectively.

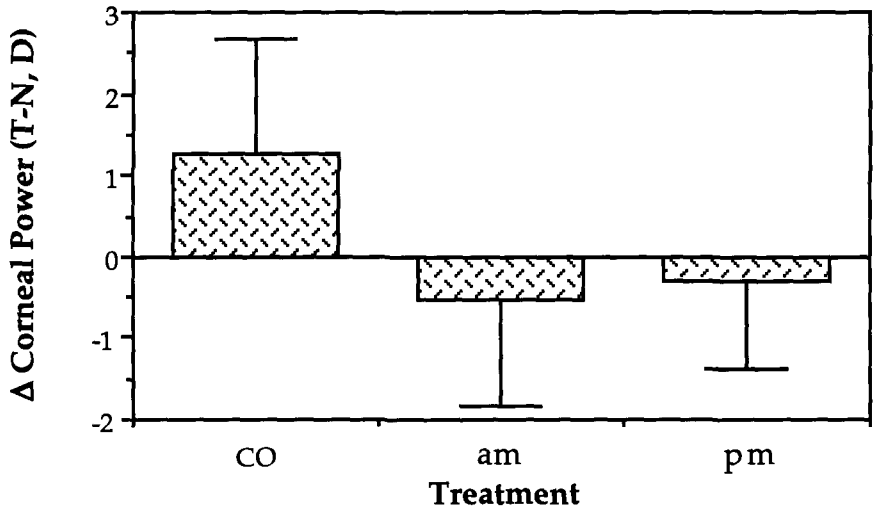


Figure 3.2.4. Differences in corneal power between treated (T) and normal (N) eyes, at day 10, for constant occlusion (CO), and interrupted occlusion groups (am, pm; mean ± SE). There was no significant difference between treatment groups at $P < 0.05$, Mann-Whitney U-test.

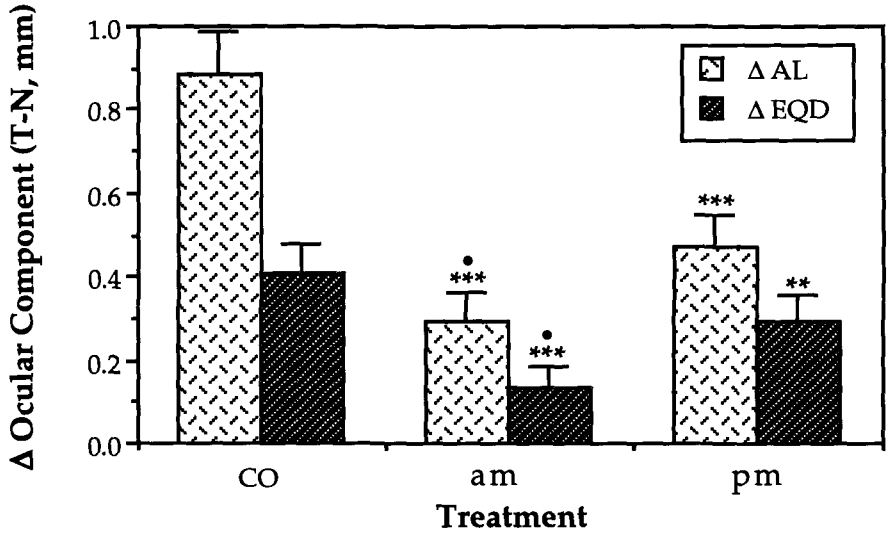


Figure 3.2.5. Differences at day 10, in externally measured axial length (AL) and equatorial diameter (EQD) between treated (T) and normal (N) eyes, for constant occlusion (CO) and interrupted occlusion groups (am, pm; mean ± SE). Differences between CO and interrupted occlusion treatment groups significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ Mann-Whitney U-test (one-tailed), differences between am and pm treatment groups significant at •$P < 0.05$, ••$P < 0.01$, •••$P < 0.005$, (MWUT, two-tailed).

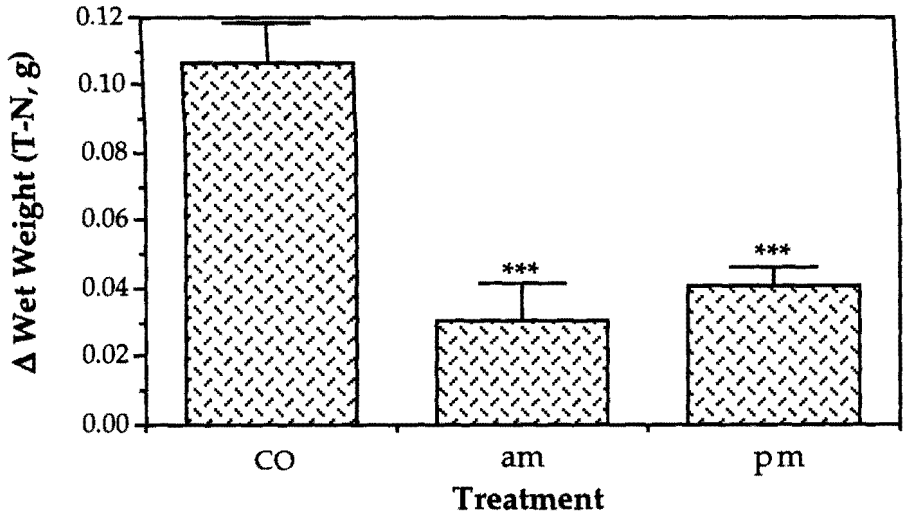


Figure 3.2.6. Differences in eye weight between treated (T) and normal (N) eyes, at day 10, for constant occlusion (CO) and interrupted occlusion groups (am, pm; mean ± SE). Differences between CO and interrupted occlusion treatment groups significant at *P < 0.05, **P < 0.01, ***P < 0.005 Mann-Whitney U-test (one-tailed). There was no significant difference between the am and pm treatment groups.

3.2.4. Discussion

Morning compared with afternoon vision

Brief periods of normal vision in the morning were as effective as periods in the afternoon at preventing form-deprivation myopia and deprivation-induced axial elongation. A four fold greater myopic shift in refractive error and a more than two fold greater increase in axial length, were recorded for the constant compared with interrupted occlusion treatment groups. Deepening of the anterior chamber, characterizing constant occlusion, was resistant to the effects of brief periods of normal vision. This is similar to the pattern of anterior chamber recovery reported previously (sections 2.1, 2.2, 2.3), where deprivation-induced anterior chamber deepening increased during the first week of normal vision, thus indicating resilience over a much longer time scale.

Comparison to predictions

It was predicted, based on the data of Weiss and Schaeffel (1993) showing that a night time growth phase could be attributed to occlusion-induced myopia, that periods of normal vision in the afternoon would be more effective than those in the morning at preventing both occlusion-induced myopia and axial expansion due to a greater inhibitory effect on the night time period of anomalous growth. However, the results obtained do not support this hypothesis; no differences relative to the timing of normal vision were observed. Two different explanations for the negative result are proposed: i) the diurnal patterns in ocular growth for the two interrupted occlusion treatment groups are identical, with both resulting in a decrease in amount of anomalous night-time growth, or ii) the period of normal vision acts to decrease the rate of ocular growth below normal, due to the effect of choroidal swelling (Wallman *et al.*, 1994; section 3.1), for a finite time period which is tied to the duration of normal vision and not its timing. The second explanation is the more plausible for the following reasons: i) as constantly occluded eyes grow during both the day and the night a period of decreased ocular growth would result in less myopia irrespective of its timing, ii) it has been shown in myopia recovery experiments that once normal vision is restored, axial eye growth effectively ceases until a normal refractive error is obtained (reviewed in Wallman, 1991; Chapter 2), and iii) results reported elsewhere suggest (section 3.1) that periods of normal vision continue to affect eye growth even when occluders are replaced.

Significance for future experiments

The data indicate that equivalent durations of normal vision on a daily basis offer equivalent "protection" from myopia irrespective of the timing of the period of normal vision. This important finding was used in designing other "follow-up" experiments which use either interrupted-occlusion or interrupted-lens-wear paradigms and where, for practical reasons, the timing of the period of visual stimulation was varied by up to five hours for different chicks.

3.2.5. Conclusion

In conclusion, periods of normal vision given in the morning, i.e. at the start of the light cycle are as effective as those given in the afternoon, i.e. at the end of the light cycle, at preventing occlusion induced myopia and axial elongation.

3.3. Sensitivity to Refractive Defocus

3.3.0. Summary

The ability of the chick eye to detect and respond to critical levels of defocus, was investigated by the application of +1 D and –1 D spectacle lenses. The 2 D difference in refractive power of the lenses induced differences in refraction between the two eyes of 2.13 D by day 6 and differences in vitreous chamber depth and axial length of 0.09 mm and 0.09 mm respectively. These data suggest that the eyes of young chicks are able to respond to this low level of refractive defocus, even though it is similar in magnitude to the estimated depth-of-focus of the eye and would thus not be expected to cause significant blurring of the retinal image.

3.3.1. Introduction

The visual system relies on information in the retinal image for accuracy of the emmetropization response (reviewed in Wallman, 1991 and Medina, 1993). This information is limited by the optics of the eye and the resolving power of the retina. One important optical factor is the depth-of-focus of the eye, which is a measure of the magnitude of defocus of the retinal image that is required for the image to be detectably blurred. The functional depth-of-focus depends upon the test conditions, pupil size and acuity (Tucker and Charman, 1975). Widely varying values of ±0.63 D to ±0.94 D (Ogle and Schwartz, 1959), ±0.38 D to ±0.66 D (Schwartz and Ogle, 1959), ±1.2 D (Tucker and Charman, 1975) and ±0.16 D to ±0.47 D (Charman and Whitefoot, 1977) have been reported for adult humans. The wide variations may be due to differences in test stimuli or to differences in the criterion used for just detectable blur. As pupil size decreases the depth-of-focus increases and large levels of defocus can be

tolerated; the accommodation system of humans responds to this situation by tending towards its resting state (Ward and Charman, 1985).

The chick eye is much smaller than the human eye and also has a smaller pupil. These factors contribute to the larger depth-of-focus in the chick eye. The depth-of-focus of the chick eye can be estimated from its ocular dimensions and visual acuity (Green *et al.*, 1980). The central, high performance areas of retina can detect lower levels of blur than the periphery and may be most important for emmetropization. While partial occlusion studies (Wallman *et al.*, 1987) show that the periperal retina responds to form deprivation, there is no equivalent lens study indicating that this is the case for refractive defocus. Visual acuities of 12.9 cycles/degree and 1.5 cycles/degree for the chick are obtained from central peak ganglion cell counts of the chick eye (Ehrlich, 1981), and from behavioural studies (Over and Moore, 1981), respectively. The higher acuity limit gives a smaller depth-of-focus value compared with that derived from the coarser behavioural acuity limit. For a pupil diameter of 2 to 3 mm the chick eye will theoretically have a depth-of-focus of at least ±0.75 D to ±1 D and the depth-of-focus may be as great as ±1.5 to ±2 D (Fig. 3.3.1). The true depth-of-focus of the chick eye probably lies somewhere between the two calculations, with the behavioural data over-estimating the actual depth-of-focus and the anatomical data under estimating it. The above estimates imply that the retinal image would have to be out-of-focus by at least 0.75 D to 1.00 D before the defocus would be detected by the retina. Using the same calculation technique Green *et al.* (1980) estimated that the depth-of-focus of the adult human eye was ±0.1 D.

It has been shown, that chicks raised with ophthalmic lenses in front of their eyes can make the appropriate eye growth adjustment to compensate for the imposed defocus (Schaeffel *et al.*, 1988). For example, chick eyes made functionally myopic, by positive lenses, tend to grow towards hyperopia and eyes made functionally hyperopic, by negative lenses, tend to grow towards myopia. The shifts in refractive state are always in the direction which compensates for the defocus provided by the lenses. The studies of Schaeffel *et al.* (1988) and Irving *et al.* (1991), both used moderate to high powered spectacle lenses, ranging in power from 4 D to 15 D.

How does the eye respond to critical levels of defocus, i.e. when the magnitude of the induced change in focus is within the eye's own depth-of-focus? The study described in this section investigated the effect of low powered spectacle lenses (+1 D and −1 D) on eye growth. As a control for

the effect of lens wear *per se* on ocular growth, some chicks were fitted with zero powered (plano) spectacle lenses. The working hypothesis was that ocular growth would not compensate for defocus levels less than the eye's own depth-of-focus. To determine if this was the case, chicks were raised with low powered spectacle lenses.

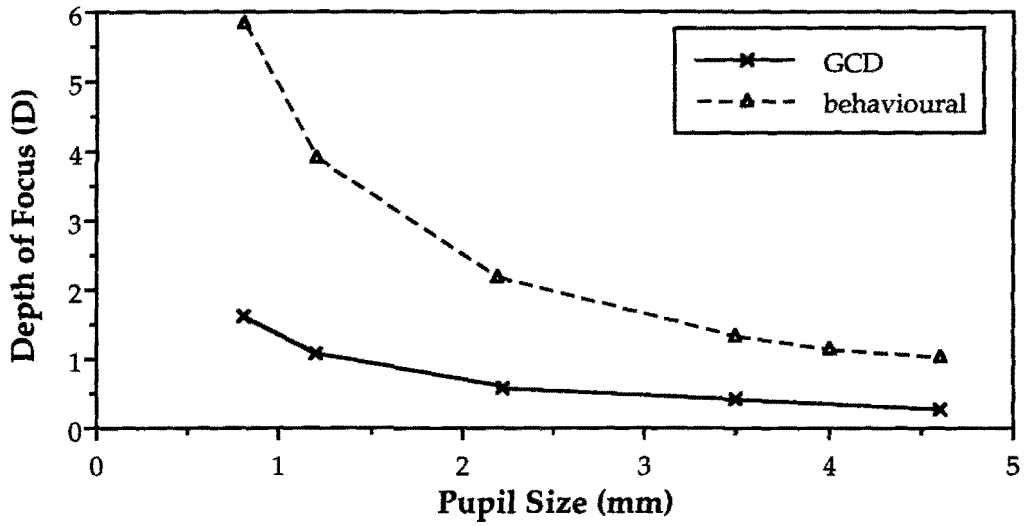


Figure 3.3.1. The depth of focus of the chick eye derived from the peak ganglion cell density (GCD) (Ehrlich, 1981) and behavioural acuity limit of the chick (Over and Moore, 1981).

3.3.2. Methods

Animals and treatments

Male White Leghorn-New Hampshire cross chicks were raised with a +1 D spectacle lens in front of one eye and a –1 D lens in front of the other eye from day 1 (n = 9). A control group of chicks were fitted with plano, i.e. zero powered spectacle lenses over one eye (n = 9). The spectacle lenses were modified human PMMA hard contact lenses, glued onto a supporting ring of velcro. All lenses had the same base curve of 8.0 mm and optic zone diameter of 11.5 mm. Chicks were reared under bright white light, of 250 lux at the level of the food trough, with a light cycle of 10 hrs light and 14 hrs dark. Food and water were provided *ad libitum*.

Measurements

Refraction and axial ocular components were measured on day 1, before lens application and then at days 6 and 9 during lens wear; corneal curvature was measured only on days 6 and 9. All measurements were performed under dim illumination. Infrared-video-photokeratometry (Schaeffel and Howland, 1987) was used to measure corneal curvature. A-scan ultrasonography (Wallman and Adams, 1987) was used to measure the anterior chamber depth (ACD), axial lens thickness (LT) and vitreous chamber depth (VCD) and static retinoscopy (non-cycloplegic) used to determine refractive errors. Measurements of refraction and axial ocular dimensions were made under halothane anaesthesia; corneal curvature was measured under ketamine/Rhompun anaesthesia (see Appendix I for greater detail).

Lenses were removed for extremely short periods, twice a day for cleaning, during which time chicks without lenses were kept in the dark. The chicks showed no detrimental behavioural effects attributable to lens wear.

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and control eyes of the same animal, the Wilcoxon matched-pairs signed-rank test (WSRT) was used. To assess the difference between +1/−1 D and plano/normal treatment groups, the Mann-Whitney U-test (MWUT) was used (see Appendix I for more details). Differences between day 6 and day 1 data were used as an index of ocular growth. In the results section data are reported as mean ± SD unless otherwise stated.

3.3.3. Results

Effects of low levels of defocus

Refractive adaptation to the low powered spectacle lenses occurred by the first measurement point, at day 6. The 2 D difference in refractive power of the lenses induced differences in refraction between the two eyes of 2.13±1.0 D, at day 6 ($P < 0.01$, WSRT, Table 3.3.1). Those eyes that wore the +1 D lens exhibited hyperopic refractive errors of +5.0±0.6 D at day 6,

which was very similar to that measured at day 1, i.e. +5.3±1.3 D (Fig 3.3.2); thus the +1 D lens tended to inhibit the normal decrease in hyperopia with age. A decrease in the hyperopia from that measured at day 1 did occur in those eyes wearing the –1 D lens; hyperopia decreased to +2.9±1.0 D in this case. Results at day 9 were similar, although the interocular refractive difference decreased to a level where it was no longer significant.

The difference in refraction was primarily due to those eyes experiencing hyperopic defocus having longer vitreous chambers, and thus ALs, than those experiencing myopic defocus. At 6 days, there was a 0.09±0.05 mm difference in both VCD and AL between these eyes (VCD and AL, $P < 0.05$, WSRT, Fig. 3.3.2 and Fig. 3.3.3); this difference had increased to 0.10±0.03 mm and 0.11±0.03 mm, respectively (VCD and AL, $P < 0.05$, WSRT) by day 9. There was no significant interocular difference in ACD, ALT (Fig. 3.3.3) or corneal power (Fig. 3.3.4) at either day 6 or day 9.

Plano lens effect

For the group that was fitted with only one plano lens, lens wear resulted in shifts in refraction in the same direction as for the positive lens. Slight, but not significant hyperopic shifts in the refraction of treated (+3.2±1.3 D) compared with normal eyes (+2.2±0.6 D) were measured at day 6 (Fig. 3.3.5). However, unlike +1 D lens wear, hyperopia, as measured at day 1, decreased in the presence of the plano lens. At day 6, the refractions of normal eyes, i.e. eyes with no lenses, were relatively more myopic than predicted based on assumptions that: i) their refractive errors should be similar in magnitude to those eyes wearing the plano spectacles lenses, and ii) their refractive errors should lie between those for the +1 D and –1 D lenses. Results at day 9 were similar, although the refractive difference between plano and no lens eyes had greatly decreased to 0.39±0.7 D.

There was no significant interocular difference for any of the measured ocular parameters, at both days 6 and 9, although there was a trend for the plano lens treatment to produce flatter corneas, shallower VCDs and shorter ALs compared to the normal contralateral eye. Interocular differences in VCD and AL were significantly greater ($P < 0.05$, MWUT) for the +1 D/–1 D compared with plano/normal treatment groups at day 9 (Table 3.3.1).

Table 3.3.1. Interocular differences in ocular parameters of chicks fitted with either +1 D and –1 D spectacle lenses (+1 D/ –1 D) or a plano spectacle lens (plano/ normal), at days 6 and 9 (mean ± SD, +1 D/–1 D, n = 9, 8; plano/normal, n = 9, 7).

Ocular parameter	+1 D/ –1 D		plano/ normal	
	day 6	day 9	day 6	day 9
Δ Refraction (D)	+2.13±1.0**	+0.83±0.3	+1.0±1.2	+0.39±0.7
Δ Corneal power (D)	+0.27±4	–1.06±2.2	–1.8±2.4	–1.6±2.7
Δ Anterior chamber depth (mm)	+0.002±0.02	–0.003±0.01	+0.02±0.01	–0.03±0.01
Δ Axial lens thickness (mm)	0.00±0.01	0.00±0.01	0.00±0.01	0.01±0.01
Δ Vitreous chamber depth (mm)	–0.09±0.05*	–0.10±0.03*	–0.06±0.11	–0.05±0.04•
Δ Axial length (mm)	–0.09±0.05*	–0.11±0.03*	–0.05±0.12	–0.07±0.04•

*Differences between eyes significant at *P < 0.05, ** P < 0.01, ***P < 0.005, Wilcoxon matched-pairs test (one-tailed). Differences between +1 D/ –1 D and plano/ normal treatment groups significant at •P < 0.05, •• P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).*

Ocular growth from day 1 to day 6

Lens wear in general disrupted the normal emmetropization process. With all lens treatments there were less than normal reductions in hyperopia from day 1 to day 6 (Fig. 3.3.8). This effect was greatest for the +1 D lens, least for the –1 D lens and intermediate for the plano lens. Axial eye growth was also affected, with all lens-wearing eyes showing reduced growth; the –1 D lenses had the least effect on growth.

Of all the measured ocular parameters, the lens grew the most over the period between measurements, with approximately a 0.28 mm increase in axial lens thickness shown by all eyes. In fact, the measured increases in ALT during this time were even greater than the AL changes. The increase in ALT appeared to be unaffected by spectacle lens wear. ACD also increased during this time, albeit to only a small degree. The anterior chamber growth was not affected by plano lens wear and was

only slightly inhibited for the other lens treatments. Over this same period, the vitreous chamber appeared to "shrink" for all lens-wearing eyes and also normal eyes. A decrease in VCD of approximately 0.05±0.09 mm was observed from days 1 to 6 for normal eyes. The "shrinkage" was even greater for the lens treated eyes; decreases in VCD of approximately 0.16±0.09 mm were measured for both the plano and +1 D lens treatments. The "shrinkage" was much less for the –1 D lens treatment, being of the order of 0.06±0.08 mm. The apparent shrinkage in VCD is assumed to be artifactual, reflecting the expansion of the more rapidly growing lens into the more slowly growing VCD.

Relative differences in vitreous chamber growth of lens wearing eyes compared with normal eyes accounted for 82%, 72% and 29% of the change in axial eye growth compared with normal, for the plano, +1 D and –1 D treatment groups respectively. Differences in the anterior chamber contributed 18%, 30% and 57% to the change in axial eye growth compared with normal for the plano, +1 D and –1 D treatment groups respectively.

Comparison to schematic eye predictions

Refractive predictions, using a schematic eye model and measured differences in the ocular parameters, only poorly correlated with the actual measured changes in refraction for both treatment groups. This was probably due to the very small differences involved. Differences in VCD contributed most to the refractive difference for the +1 D/–1 D treatment group; differences in corneal power contributed most for the plano/normal group.

Table 3.3.2. Predicted (based on ocular parameter differences) compared with measured changes in refractive error (RE) for +1 D/–1 D and plano/normal treatment groups at days 6 and 9 (mean ± SD,+1 D/–1 D, n = 9, 8; plano/normal, n = 9, 7).

	+1 D/ –1 D		plano/ normal	
	day 6	day 9	day 6	day 9
Measured Δ RE (D)	+2.13±1.0	+0.83±0.3	+1.00±1.2	+0.39±0.7
Δ RE ACD (D)	–0.06	+0.09	–0.58	+0.87
Δ RE VCD (D)	+1.42	+1.58	+0.95	+0.79
Measured Δ CP (D)	+0.27±4	–1.06±2.2	–1.8±2.4	–1.6±2.7
Predicted Δ RE (D)	+1.1	+2.7	+2.1	+3.3

Based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

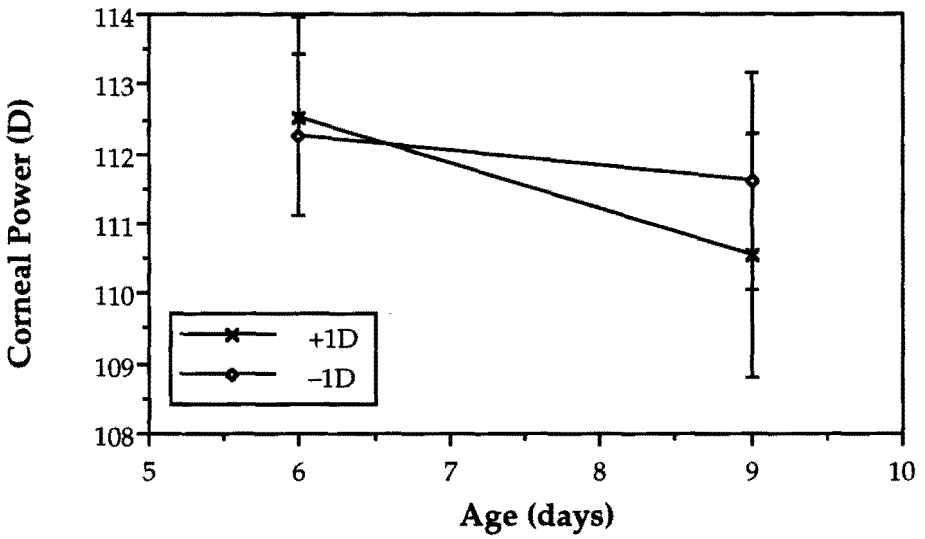


Figure 3.3.4. Corneal power (mean ± SE) of eyes experiencing myopic defocus, (i.e. wearing a +1 D lens), compared with hyperopic defocus (i.e. wearing a –1 D lens). There was no significant differences between eyes at $P < 0.05$ Wilcoxon matched-pairs signed-ranks test.

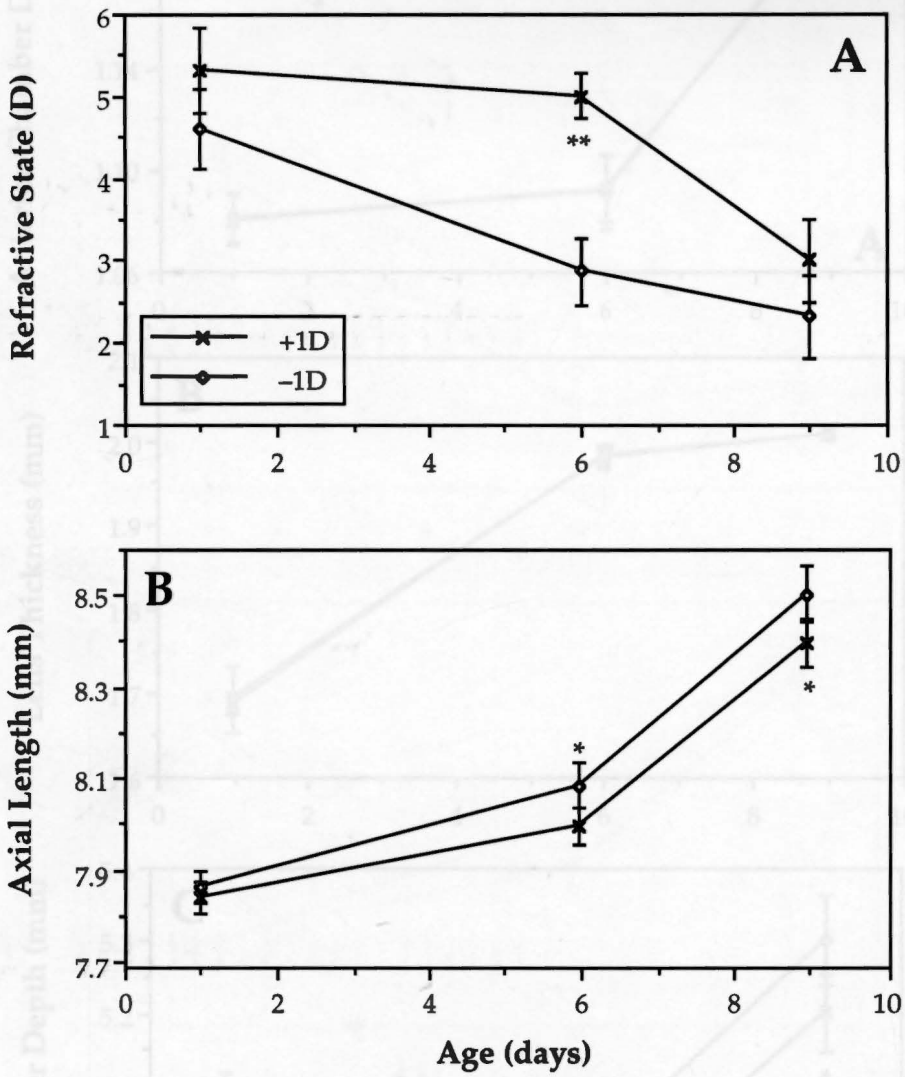


Figure 3.3.2. Refraction **(A)** and axial length **(B)** (mean ± SE) of eyes experiencing myopic defocus (i.e wearing a +1 D lens), compared with hyperopic defocus (i.e. wearing a –1 D lens). Differences between eyes significant at *P < 0.05, ** P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).

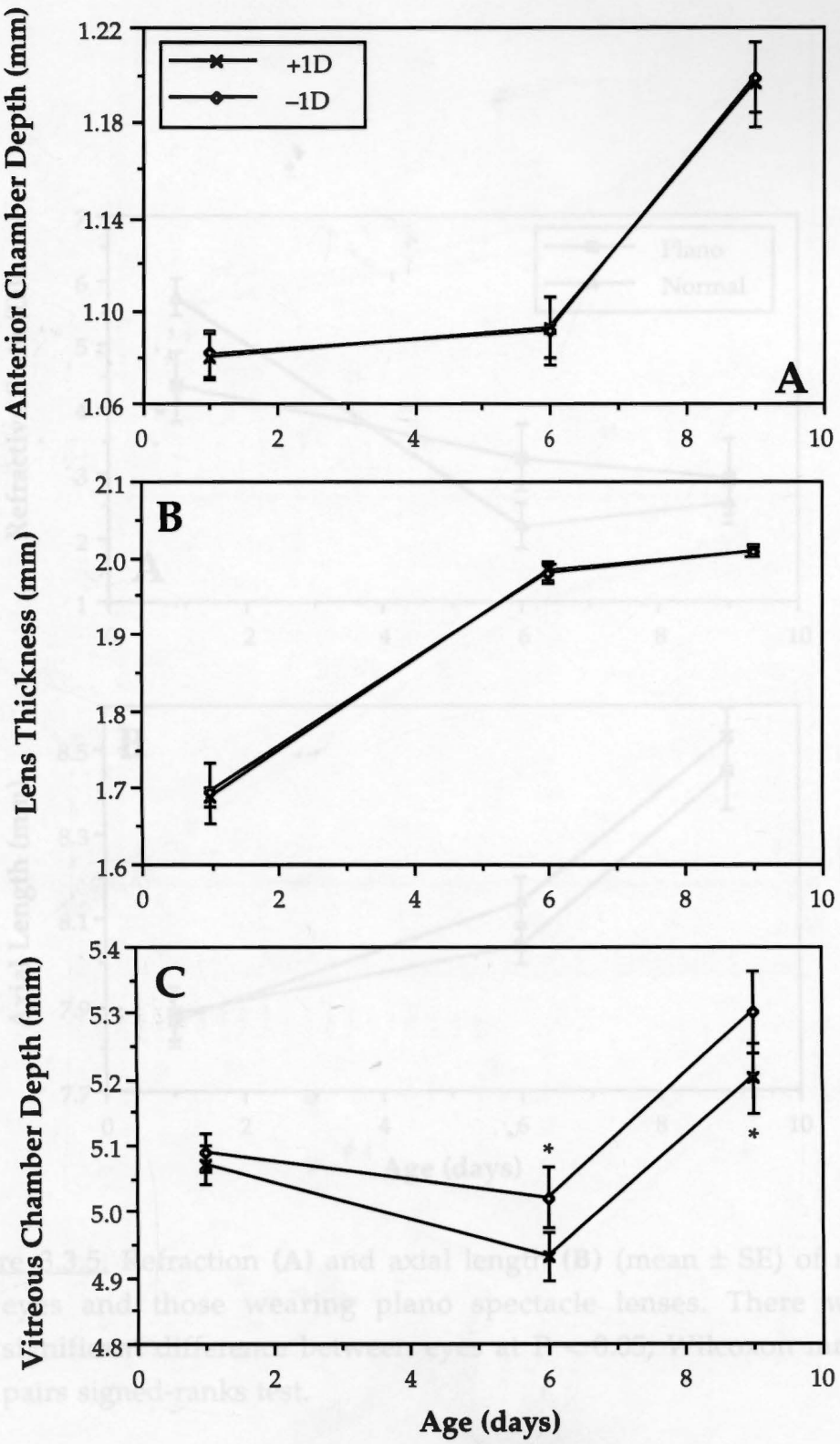


Figure 3.3.3. Anterior chamber depth, **(A)** lens thickness **(B)** and vitreous chamber depth **(C)** (mean ± SE) of eyes experiencing myopic defocus (i.e wearing a +1 D lens), compared with hyperopic defocus (i.e. wearing a –1 D lens). Differences between eyes significant at *P < 0.05, ** P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).

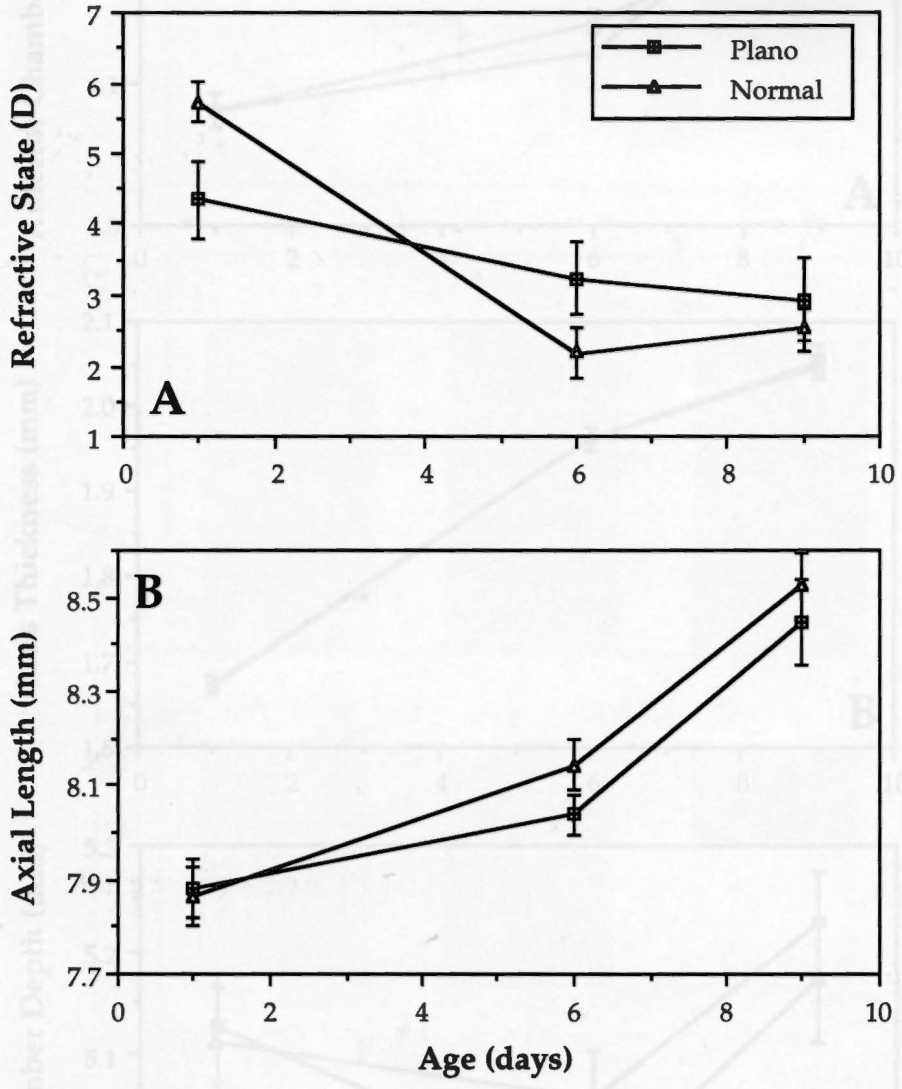


Figure 3.3.5. Refraction **(A)** and axial length **(B)** (mean ± SE) of normal eyes and those wearing plano spectacle lenses. There was no significant difference between eyes at $P < 0.05$, Wilcoxon matched-pairs signed-ranks test.

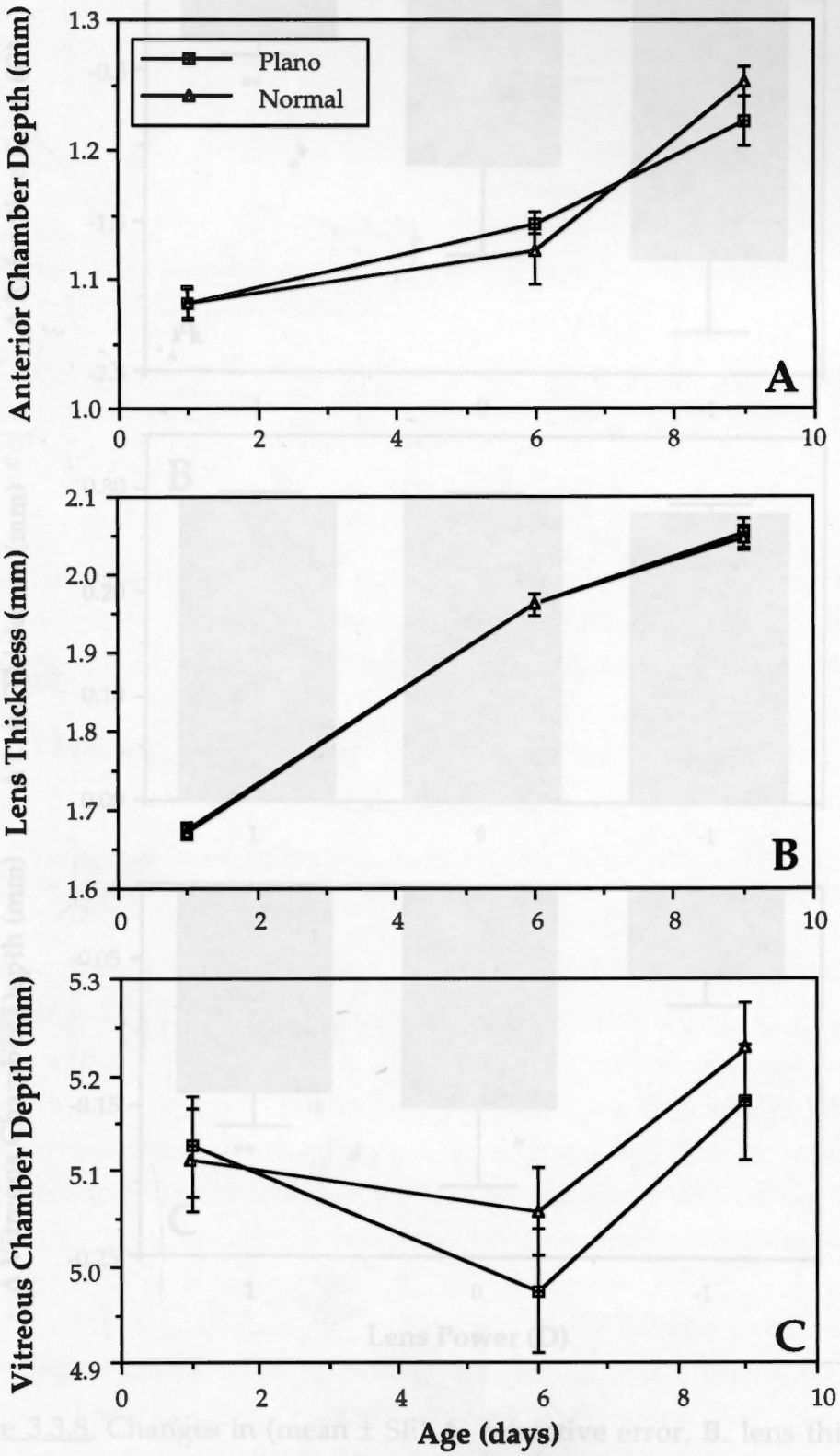


Figure 3.3.6. Anterior chamber depth **(A)**, lens thickness **(B)** and vitreous chamber depth **(C)** (mean ± SE) of normal eyes and those wearing plano spectacle lenses. There was no significant difference between eyes at $P < 0.05$, Wilcoxon matched-pairs signed-ranks test.

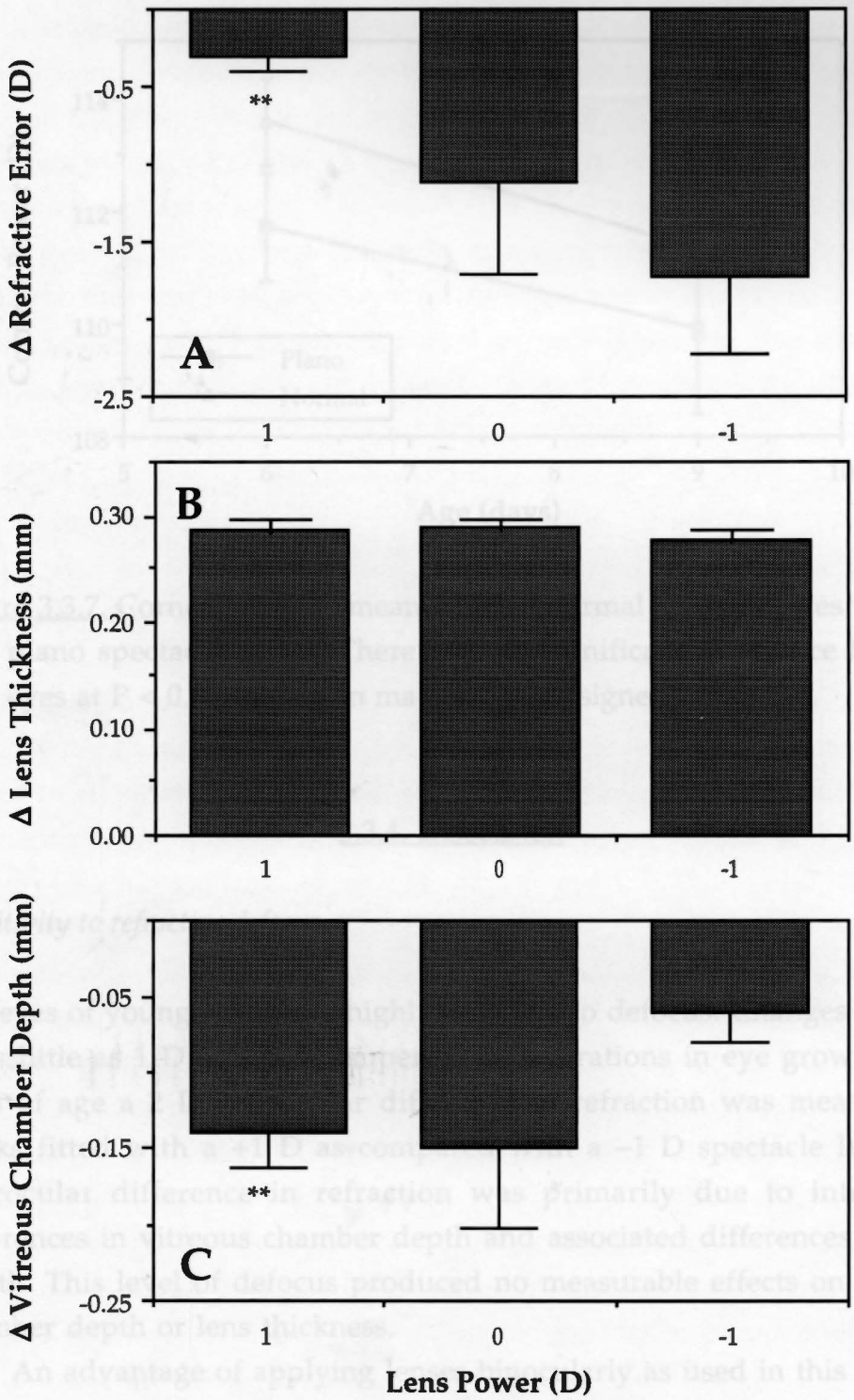


Figure 3.3.8. Changes in (mean ± SE) **A.** refractive error, **B.** lens thickness and **C.** vitreous chamber depth from days 1 to 6 for +1 D, –1 D and plano spectacle lens wear. Differences between +1 D and – 1 D lens wear significant at *P < 0.05, ** P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).

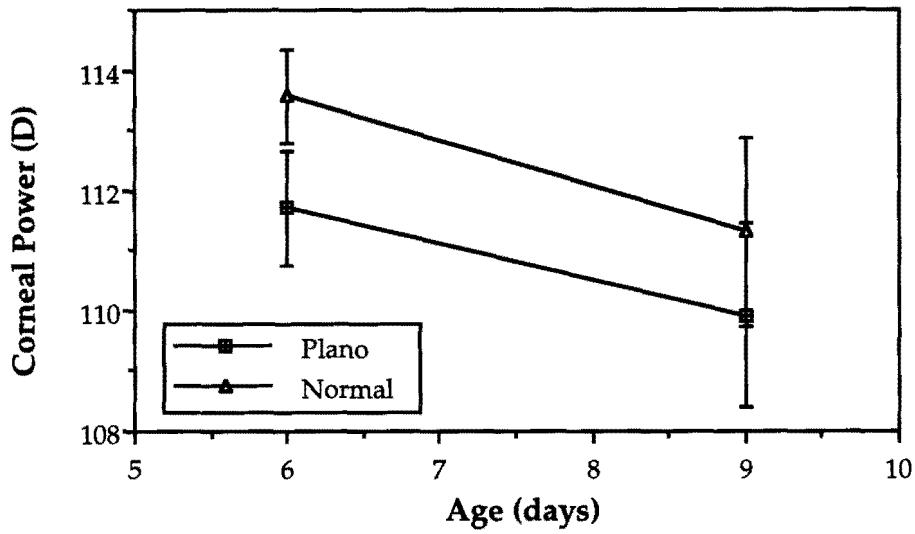


Figure 3.3.7. Corneal power (mean ± SE) of normal eyes and eyes wearing plano spectacle lenses. There was no significant difference between eyes at $P < 0.05$, Wilcoxon matched-pairs signed-ranks test.

3.3.4. Discussion

Sensitivity to refractive defocus

The eyes of young chicks are highly sensitive to defocus. Changes in focus of as little as 1 D induced compensatory alterations in eye growth. By 6 days of age a 2 D interocular difference in refraction was measured in chicks fitted with a +1 D as compared with a –1 D spectacle lens. The interocular difference in refraction was primarily due to interocular differences in vitreous chamber depth and associated differences in axial length. This level of defocus produced no measurable effects on anterior chamber depth or lens thickness.

An advantage of applying lenses binocularly as used in this study is the reduction of inherent variability between animals, this is especially important here where the effects of lens wear are small. This is a similar strategy to that used by Schaeffel *et al.* (1988) in their lens studies.

Plano lens effect

Wearing a zero powered spectacle lens resulted in slight, though not significant, hyperopic shifts in refraction compared with normal. While

this treatment provides a measure of the effect of wearing lenses *per se*, the addition of refractive power would be expected to induce additional changes from this baseline, and any measured interocular refractive difference for the +1 D and –1 D spectacle group would still be a result of the refractive difference of the lenses. The reason for the slight change in ocular growth with plano lenses is unclear; while it is interesting to speculate that the changes occurred in response to slight reductions in retinal image quality caused by dust accumulation on the lenses, the changes were in the opposite direction to those predicted by this model, i.e. hyperopic rather than myopic shifts in refraction.

Effect of depth-of-focus

The depth-of-focus of the chick eye was estimated to be between ±0.75 D and ±2 D in magnitude, depending on the visual acuity value used in its determination. On this basis it may be assumed that the retinal image would have to be out of focus by at least this amount before the defocus would be detected by the retina. For this same reason, it is predicted that compensatory responses to spectacle lens defocus would be limited by depth-of-focus leading to errors of between 0.75 D and 2 D. By extrapolation, lens powers as small as +1 D and –1 D should be within the depth-of-focus limits and, thus, little or no measurable change in ocular growth and refraction is predicted (Fig 3.3.9). That adaptation to spectacle lenses of this magnitude did occur implies that either: i) the depth-of-focus was over estimated, or ii) defocus cues are available that are not depth-of-focus limited. The latter alternative seems the more plausible as normal chicks after development do not have a residual 1 D hyperopic refractive error and eyes recovering from form-deprivation myopia do not retain 1 D of myopia (section 2). In fact, emmetropizing to one extreme of the eye's depth-of-focus would be totally inappropriate as it would make the image more susceptible to errors of focus.

This effect has a parallel in humans where it has been demonstrated that the sensorimotor threshold for the accommodation system is smaller than the depth-of-focus of the eye (Kotulak and Schor, 1986a). When changing fixation from a distance to near object, blur is not usually reported. Similarly the microfluctuations of accommodation are not perceived under normal conditions. Presumably there are higher order, e.g. cortical mechanisms that are used to dampen the effects of "blur". Similarly, the human eye is able to use longitudinal chromatic aberration

to guide accommodation even though the colour fringes produced by longitudinal chromatic aberration are not perceivable (Ogboso and Bedell, 1987). These findings support the view that the low levels of spectacle-induced defocus were detected and compensated for by the chick emmetropization system even though they were not expected to be detectable by the retina. Based on traditional depth-of-focus grounds pertaining to presumed resolution limits of eyes the results could mean that resolution *per se* is not important for defocus detection due to other changes, e.g. changes in spatial frequency profiles or contrast, and it is these changes which are detected by the retina. The results demonstrate that the eye growth control system is more sensitive than the expected resolution capacity. This also leads to the suggestion that the emmetropization system uses a system highly sensitive to blur.

It has also been suggested that the magnitude and accuracy of accommodation needed to maintain clear imagery depends on the magnitude of the eye's depth-of-focus (Green *et al.*, 1980). The accuracy of accommodation in infants improves with development (Banks, 1980), i.e. as depth-of-focus decreases (Green *et al.*, 1980).

While it is suggested here that +1 D and –1 D spectacle lenses induce low levels of defocus in the chick, a human with an uncorrected prescription of –1 D would only achieve a distance visual acuity of approximately 6/18 (Hirsch, 1945; Thorn and Schwartz, 1990) and would consider their distance blur to be significant. This effect may be due to the lesser depth-of-focus of the human eye; it has been suggested that refractive errors only become significant when greater than the depth-of-focus (Green *et al.*, 1980). This idea is reinforced by the observation that uncorrected myopes report clearer vision in bright conditions, when pupil size is reduced and depth-of-focus increased. Alternatively, due to higher order processing that is carried out on the image by the lateral geniculate nucleus and cortex, perceived blur may be less than retinal blur under some circumstances.

If in contrast to the findings of this investigation depth-of-focus has a bearing on the emmetropization response to lens defocus, it would be expected that accuracy would be improved following surgical techniques, e.g. ciliary nerve section which cause pupil dilation and hence reduce the eye's depth-of-focus; this issue is investigated further in following experiments.

Role of accommodation

Could accommodation somehow account for the changes observed in response to +1 D and –1 D lenses? Young chicks possess high levels of accommodation (Schaeffel *et al.*, 1986), and in this respect their eyes are not consensually linked. Thus images viewed through spectacle lenses of different magnitudes fitted to each eye can be simultaneously in focus. The normal refractive error of the chick at day 1 is approximately +3 D to +5 D hyperopic, and thus in the case of the +1 D/ –1 D lens treatment, the plus lens will have required accommodation to be relaxed slightly relative to the other eye with the minus lens which would have required increased accommodation for focussing at equivalent distances. These requirements seem well within the capacity of the chick's accommodation system; the likely effect of the lenses would be to create a small accommodative imbalance between eyes. However, the chick eye is able to compensate for refractive defocus in the absence of accommodative function (Schaeffel *et al.*, 1990; section 4.1) and it would thus seem unlikely that the presumed small changes in accommodative level caused the measured changes in refraction observed.

3.3.5. Conclusions

In conclusion, the eyes of young chicks are extremely sensitive to defocus, spectacle lenses with powers as low as +1 D and –1 D inducing compensatory changes in ocular growth.

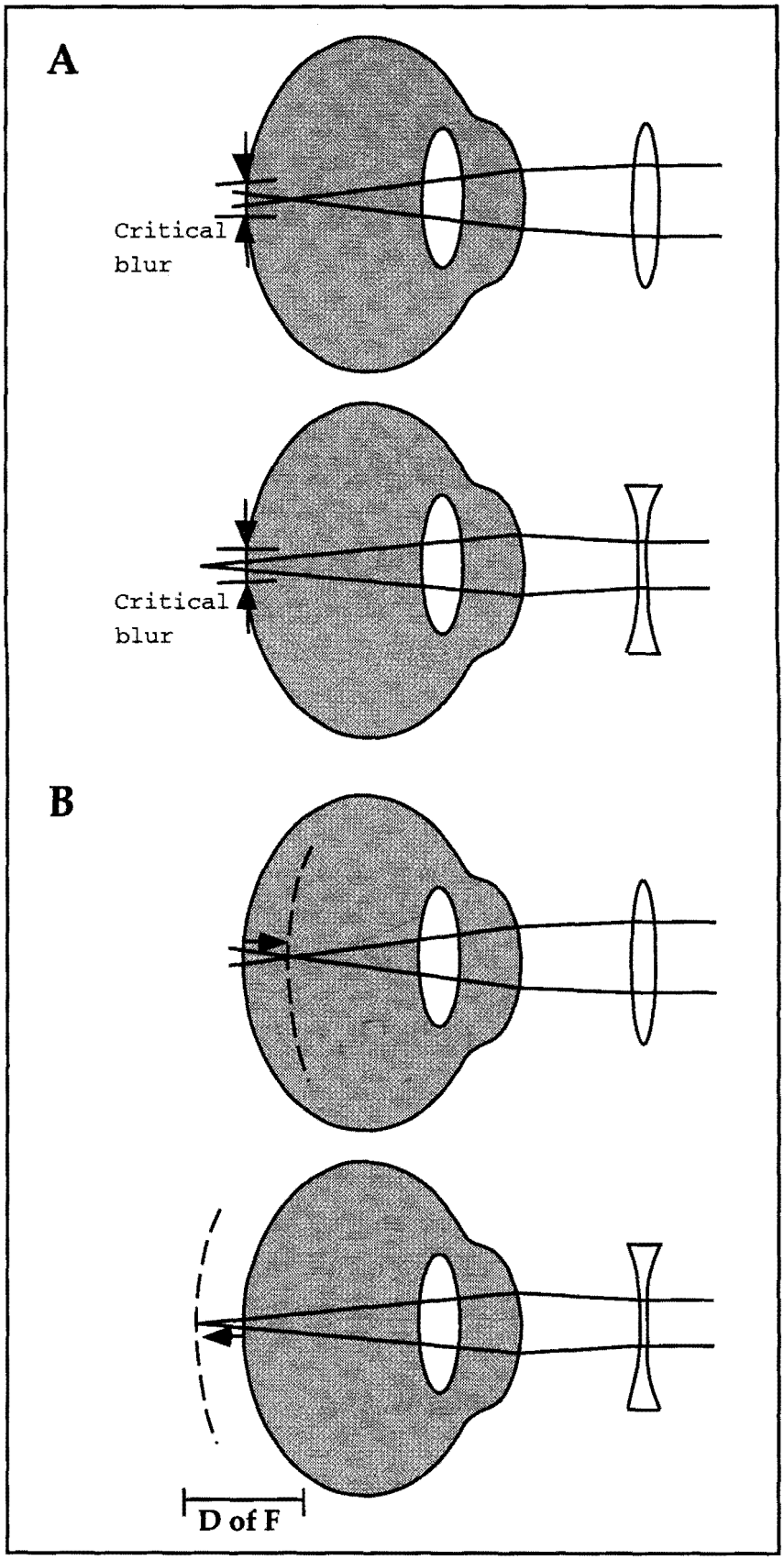


Figure 3.3.9. Predicted effects of low powered spectacle lenses on ocular growth. In **A.** defocus produces less than the critical level of blur and no changes in eye growth are seen and in **B.** ocular compensation occurs irrespective of the eyes depth-of-focus (D of F).

3.4. Normal Vision and Refractive Adaptation

3.4.0. Summary

The question of whether, like form-deprivation myopia, lens-induced myopia is prevented by brief periods of normal visual stimulation was investigated. At the same time, the effect of short periods of normal visual stimulation on the development of hyperopia in response to positive lenses was also studied. Chicks were fitted with a spectacle lens (+10 D, –10 D, or 0 D) from day 2 to day 10. Lenses were worn either constantly (0) or lens wear was interrupted with a period of normal visual stimulation (3, 6, 9, or 11 hrs per 12 hr day). Constant lens wear induced adaptational ocular growth responses which were determined by both the sign and magnitude of the induced defocus. Significant hyperopia was observed with the +10 D lenses (+8.3 D at day 5 and day 10); conversely, the –10 D lenses produced myopia (–1.6 D at day 5; –6.2 D at day 10). As in the case of form-deprivation myopia, even brief daily exposure to normal vision prevented the development of myopia in response to –10 D spectacle lenses. By contrast, hyperopia was always seen with +10 D lenses, although the magnitude of hyperopia decreased with increased duration of normal visual stimulation; average changes in refraction at day 10 of +5.8 D, +3.8 D, +3.1 D and +3.0 D were recorded for the 3, 6, 9, and 11 hr treatment groups respectively. In all cases, refractive changes largely reflected altered vitreous chamber depth, myopia and hyperopia corresponding to longer and shorter than normal vitreous chambers respectively. The results suggest that there are both "go" and "stop" signals for ocular growth that are activated by hyperopic (–10 D) and myopic defocus (+10 D) respectively. For competing "go" and "stop" growth signals, the "stop" signal dominates, so that growth is reduced in proportion to the duration of the "stop" signal.

3.4.1. Introduction

A variety of species are born with refractive errors that tend to diminish with time. In chicks highly variable, usually hyperopic, refractions move toward emmetropia with normal eye development (Wallman *et al.*, 1981); an emmetropization process is conjectured to be directing growth towards this endpoint. When a normal visual input is prevented either by lid

suture or translucent occluders, emmetropization is disrupted and excessive axial eye growth and myopia are produced (Wallman *et al.*, 1978b).

As previously alluded to, (section 3.3) altered ocular growth also occurs in response to an artificially induced refractive error. Schaeffel *et al.* (1988) found that when chicks wore either negative or positive spectacle lenses, ocular compensation for the imposed refractive error occurred. Using a different experimental paradigm and different designed lenses with younger chicks Irving *et al.* (1991) reported more complete adaptation to the lenses. Form deprivation and hyperopic defocus represent two alternative ways of inducing myopia in chicks and thus it might be expected that similar underlying mechanisms are involved. However, based on the differing effects of occluders and lenses on the retinal image it has been suggested that different processes underlie the form-deprivation and lens-induced effects (Schaeffel *et al.*, 1992). In further support of this idea, it has been recently reported that while 6-hydroxydopamine blocks the development of form-deprivation myopia it does not prevent the ocular adaptation to positive and negative spectacle lenses (Schaeffel *et al.*, 1992). In contrast, optic nerve section reduces the response to negative lenses while not affecting the deprivation response (Wildsoet and Wallman, 1992).

Optically, form deprivation and and negative lenses can not be considered synonymous. Optical blur results from negative spectacle lenses while translucent occluders cause reductions in image quality, i.e. reductions in contrast and spatial frequency information. Also in the case of the negative lenses image quality may be improved by accommodation. Given that accommodation in young chicks may be as high as 17 D (Schaeffel and Howland, 1987; Wallman and Adams, 1987) this is more than adequate to clear for distance vision the -10 D lenses used in the current study. Some accommodation is also left in reserve for near viewing. With the +10 D lens also used in this study, distant objects will appear blurred, although a *very* slight improvement in distance vision may be achieved if the chick eye is able to relax its tonic accommodation; tonic accommodation has been estimated as 4 D in magnitude (Troilo *et al.*, 1993). For this lens, near vision will be clear within 10 cm and achieved with a much reduced effort.

It is not known if the eye would still compensate for the refractive defocus of the lens if applied intermittently, i.e. brief daily periods of normal vision given with lens wear. Form-deprivation myopia is

extremely sensitive to periods of normal vision and can be totally prevented by brief daily periods of normal visual stimulation (Nickla *et al.*, 1989). Altered ocular growth in response to refractive spectacle lenses may respond in a similar fashion to form-deprivation myopia, i.e. no compensation when short periods of normal visual stimulation are introduced; alternately it may contrast with form-deprivation myopia and adaptation occur in proportion to the duration of lens wear. The effect of lens wear *per se* and also the effect of interrupting lens wear with variable periods of normal vision were investigated.

3.4.2. Methods

Animals

Male, White Leghorn-New Hampshire cross chicks were obtained from a local hatchery on the day of hatching. They were raised in temperature controlled enclosures with food and water provided *ad libitum*. Chicks were exposed to a 12 hr light/ 12 hr dark diurnal light cycle with lights on at 7 am and off at 7 pm. A light intensity of 250 lux at the level of the food trough was provided by overhead fluorescent lights. The experiment was run as a number of repeats so as to obtain results for 6 to 7 chicks for each experimental condition. Additional chicks were subjected to the 1 hr of wear per day paradigm where morning lens wear versus evening lens wear was compared; a total of 12 chicks were used here. Four batches of chicks (no. 21, 27, 30, and 33) were used in total, with roughly equal numbers of chicks in each batch being assigned to each lens wearing schedule and each lens power (+10 D, –10 D or plano). Approximately equal numbers of right and left eyes were used for lens wear.

Lens wearing protocol

Treatment paradigms are summarized in Table 3.4.1. Chicks were reared with either a –10 D (hyperopic defocus), +10 D (myopic defocus) or plano (control) spectacle lens (Fig. 3.4.1). Some chicks wore the lenses all day and others wore the lenses for only part of the day. Thus lenses were worn for either 1, 3, 6, 9 or 12 hrs (constant lens wear) per 12 hr day with chicks experiencing normal vision for the remainder of the day, i.e. either 11, 9, 6, 3 or 0 (constant lens wear) hrs/day. The period of normal vision was given in one complete block of time, with half the chicks always

receiving normal vision always in the morning immediately after "lights on"and the other half always in the evening prior to "lights out"; thus the period of lens wear was never split across the day. The chicks showed no adverse behavioural effects attributable to lens wear.

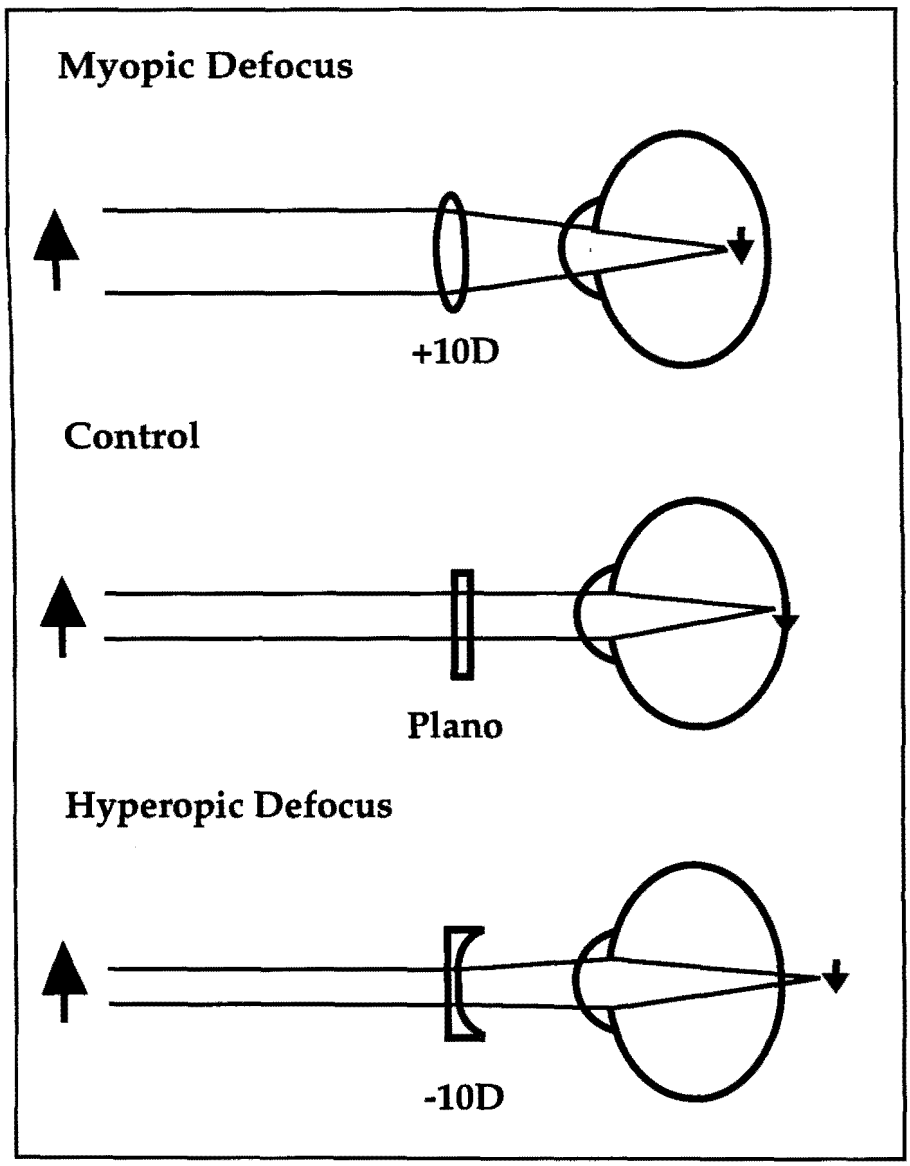


Figure 3.4.1. Effect of spectacle lenses on the vergence of light for an unaccommodated eye and object at infinity. The +10 D lens produces myopic defocus by shifting the image plane anteriorly, the plano lens has no effect on focus and the –10 D lens produces hyperopic defocus by shifting the plane of focus posteriorly.

Table 3.4.1. Treatment paradigms showing the powers of spectacle lenses used and duration of lens wear.

Spectacle lens	Defocus	Hours of lens wear (hrs/day)				
+10 D	Myopic	1 (11)	3 (9)	6 (6)	9 (3)	12 (0)
–10 D	Hyperopic	1 (11)	3 (9)	6 (6)	9 (3)	12 (0)
Plano	Control	1 (11)	3 (9)	6 (6)	9 (3)	12 (0)

Duration of normal vision bracketed.

The spectacle lenses used were modified human PMMA contact lenses with 12 mm diameters, large optic zones (10.5 mm to 11.5 mm) and 8.0 mm back optic radii. The measured vertex distance of the applied lenses was approximately 3 mm to 4 mm. Thus the effective power of the lenses at the cornea, were +10.3 D to +10.4 D, in the case of the +10 D lenses and –9.7 D to –9.6 D, in the case of the –10 D lenses. In all sections to follow, lens power is referred to without taking vertex distance into account, because of the difficulty in accurately measuring vertex distance and its variability. Chicks were checked 5 to 6 times per day to replace lost lenses or dirty lenses; dirty lenses were replaced with a clean lens of the same power, the thumb or index finger being placed over the chicks' eye to prevent normal vision during the exchange (see Appendix I for further information on the lenses).

Measurements

Ocular measurements were made on both days 5 and 10. Chicks were anaesthetized using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed in a dim room to determine the refractive error and the internal axial dimensions respectively. Anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987), under ketamine/Rhompun anaesthesia.

After the above measurements, on day 10, chicks were given an overdose of sodium pentobarbitone. The eyes were excised, cleared of extraneous muscle tissue and the external axial length and equatorial diameters measured directly with digital calipers. The eyes were also weighed (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated (T) and normal (N) eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test was used (WRST). To assess the effect of different durations of lens wear and the effect of different lens powers, the Mann-Whitney U-test was used (MWUT; see Appendix I for more detail). All data is reported as mean ± SD unless otherwise stated.

3.4.3. Results

Constant lens wear

Constant spectacle lens wear induced adaptational ocular growth responses, which varied with both the sign and magnitude of the induced defocus (Table 3.4.2, day 5; Table 3.4.3, day 10). Only plano lenses had little effect on refraction; slight hyperopia, +1.0±1.5 D was observed on day 5 and eyes were approximately emmetropic, +0.2±1.3 D, on day 10. Refractive adaptation to –10 D lenses (hyperopic defocus) occurred slowly with a –1.6±0.9 D myopic shift in refraction produced by day 5, increasing to –6.2±3.0 D by day 10. In contrast, +10 D lenses (myopic defocus) quickly produced large hyperopic shifts, +8.3±2.8 D by day 5 and +8.3±1.4 D by day 10. This difference in response rates is reflected in the significantly greater refractive change (refraction T compared with N) in response to +10 D lens wear compared with –10 D lens wear at day 5 ($P < 0.01$, MWUT); by day 10, there was no statistical difference between the +10 D and – 10 D lens groups in this respect.

The changes in refraction produced by constant lens wear were primarily due to alterations in the growth of the vitreous chamber (Table 3.4.4), with negative lenses (hyperopic defocus) increasing and positive lenses (myopic defocus) decreasing growth. Constant –10 D lens wear (hyperopic defocus) increased VCD growth by 0.15±0.07 mm and 0.27±0.09 mm relative to the normal eye at day 5 and 10 respectively. In contrast, constant +10 D lens wear (myopic defocus) slowed VCD growth by 0.29±0.14 mm at day 5 and 0.16±0.11 mm at day 10. These changes in the VCDs were highly correlated to changes in AL at both measurement points and for both treatment groups ($r = 0.965$, $P < 0.002$, +10 D, day 5; $r = 0.951$, $P < 0.005$, +10 D, day 10; $r = 0.977$, $P < 0.001$, –10 D, day 5; $r = 0.853$, $P <$

0.05, –10 D, day 10; Fig. 3.4.2). Plano spectacle lens wear did not significantly affect VCD growth.

Constant lens wear had no significant effect on ACD (Fig. 3.4.4, day 5; Fig. 3.4.6, day 10) or ALT (Fig. 3.4.3, day 5; Fig. 3.4.5, day 10), i.e. there was no difference between treated and normal eyes at both day 5 and 10 for all treatment groups. Corneal flattening was evident with constant lens wear at day 10 (Table 3.4.3; Fig. 3.4.7). This trend was observed for all lens types, irrespective of their sign, although it was most pronounced for the +10 D lens group. The +10 D lens produced 5.8±1.0 D of corneal flattening, the –10 D lens produced 2.2±2.3 D, and 3.0±2.7 D of flattening was produced by the plano lens, at day 10. This effect of lens wear was not evident in the earlier, day 5 data.

Significant differences were observed between effects of the +10 D lens and the –10 D lens on external axial lengths, as measured on day 10. The +10 D lens resulted in eyes which were on average 0.045±0.1 mm shorter than contralateral normal eyes and the –10 D lens produced a relative increase in the external axial length of 0.15±0.1 mm. There were no significant differences between treated and normal eyes with respect to wet eye weight, equatorial diameter and corneal diameter data for all lens types.

Table 3.4.2. Effect of constant lens wear on ocular parameters, at day 5. The difference between treated and normal eyes are shown (mean ± SD, n = 6, 6, 6).

Ocular parameter	+10 D (myopic defocus)	–10 D (hyperopic defocus)	Plano (control)
Δ Refraction (D)	+8.3±2.8***	–1.6±0.9**	+1.0±1.5
Δ Corneal power (D)	–1.6±3.1	–0.5±3.7	+1.4±5
Δ Anterior chamber depth (mm)	+0.03±0.09	+0.01±0.04	–0.01±0.03
Δ Axial lens thickness (mm)	–0.01±0.02	–0.02±0.02	+0.01±0.02
Δ Vitreous chamber depth (mm)	–0.29±0.16***	0.15±0.07***	–0.09±0.12
Δ Axial length (mm)	–0.28±0.17**	+0.14±0.09***	–0.09±0.11

*Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).*

Table 3.4.3. Effect of constant lens wear on ocular parameters, at day 10. The differences between treated and normal eyes are shown (mean ± SD, n = 6, 6, 6).

Ocular parameter	+10 D (myopic defocus)	−10 D (hyperopic defocus)	Plano (control)
Δ Refraction (D)	+8.3±1.4***	−6.2±3.1***	+0.2±1.3
Δ Corneal power (D)	−5.8±1.0**	−2.2±2.3	−3.0±2.7
Δ Anterior chamber depth (mm)	−0.01±0.05	+0.03±0.08	+0.01±0.04
Δ Axial lens thickness (mm)	−0.01±0.02	−0.01±0.02	−0.02±0.02
Δ Vitreous chamber depth (mm)	−0.17±0.11***	+0.27±0.09***	+0.06±0.08
Δ Axial length (mm)	−0.18±0.12**	+0.28±0.09***	+0.05±0.07

*Differences between +10 D and −10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).*

Predicted differences in refraction based on ocular parameter differences for constant lens wear

Predictions of differences in refraction based on measured differences in ACD and VCD were in reasonable agreement with those measured directly (Table 3.4.4). This analysis confirmed the significant contribution of the vitreous chamber changes to the refractive changes although, for the constant +10 D lens group, corneal flattening also contributed to the measured hyperopic shift (70% of predicted change).

Table 3.4.4. Predicted (based on ocular parameter differences) compared with measured changes in refractive error (RE) for +10 D, −10 D and plano constant lens wear treatment groups at day 5 and 10.

	+10 D		−10 D		Plano	
	day 5	day 10	day 5	day 10	day 5	day 10
Measured Δ RE (D)	+8.3±2.8	+8.3±1.4	−1.6±0.9	−6.2±3.1	+1.0±1.5	+0.2±1.3
Δ RE ACD (D)	−0.87	+0.29	−0.29	−0.87	+0.29	−0.29
Δ RE VCD (D)	+4.59	+2.69	−2.37	−4.27	+1.42	−0.94
Measured Δ CP (D)	−1.6±3.1	−5.8±1.0	−0.5±3.7	−2.2±2.3	+1.4±5	−3.0±2.7
Predicted Δ RE (D)	+5.3	+8.8	−2.2	−2.9	+0.3	+1.8

Predicted ΔRE based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

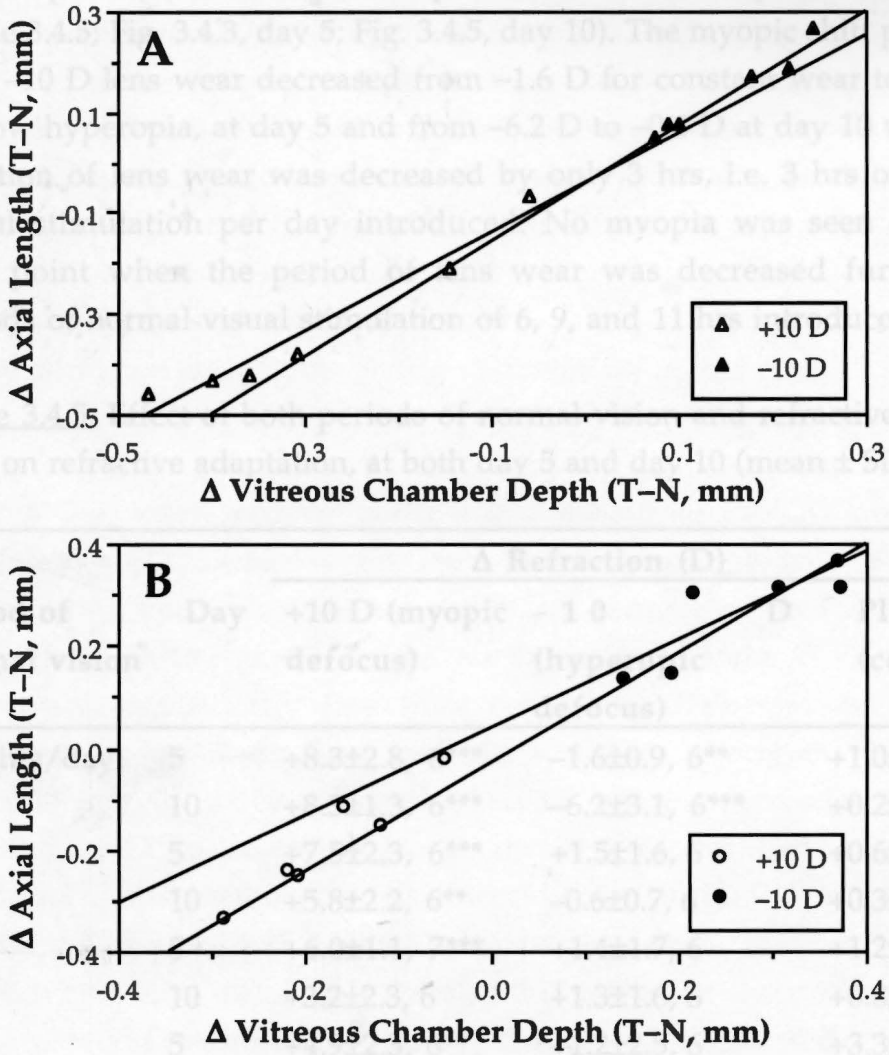


Figure 3.4.2. Relationship between differences in vitreous chamber depth (ΔVCD) of treated and normal eyes and differences in axial length (ΔAL), for +10 D and –10 D lenses (constant wear), at **A.** day 5 and **B.** day 10. ΔVCD and ΔAL were highly correlated for both the +10 D (r = 0.965, $P < 0.002$, day 5; r = 0.951, $P < 0.005$, day 10) and –10 D (r = 0.977, $P < 0.001$ day 5; r = 0.853, $P < 0.05$, day 10) treatment groups at both measurement points.

Refractive adaptation for refractive defocus and periods of normal vision

In the case of the –10 D lens, i.e hyperopic defocus, even brief periods of daily exposure to normal vision prevented the development of myopia (Table 3.4.5; Fig. 3.4.3, day 5; Fig. 3.4.5, day 10). The myopic shift produced with –10 D lens wear decreased from –1.6 D for constant wear to +1.5 D, i.e. low hyperopia, at day 5 and from –6.2 D to –0.6 D at day 10 when the duration of lens wear was decreased by only 3 hrs, i.e. 3 hrs of normal visual stimulation per day introduced. No myopia was seen at either time point when the period of lens wear was decreased further, i.e. periods of normal visual stimulation of 6, 9, and 11 hrs introduced.

Table 3.4.5. Effect of both periods of normal vision and refractive defocus on refractive adaptation, at both day 5 and day 10 (mean ± SD, n).

		Δ Refraction (D)		
Period of normal vision	**Day**	**+10 D (myopic defocus)**	**– 1 0 D (hyperopic defocus)**	**Plano (control)**
0 (hrs/day)	5	+8.3±2.8, 6***	–1.6±0.9, 6**	+1.0±1.5, 6
	10	+8.3±1.3, 6***	–6.2±3.1, 6***	+0.2±1.3, 6
3	5	+7.5±2.3, 6***	+1.5±1.6, 6	+0.6±0.7, 6
	10	+5.8±2.2, 6**	–0.6±0.7, 6	+0.3±1.2, 6
6	5	+6.0±1.1, 7***	+1.4±1.7, 6	+1.2±2.5, 6
	10	+3.2±2.3, 6	+1.3±1.6, 6	+0.8±1.2, 6
9	5	+4.9±2.3, 6**	+1.2±2.5, 6	+3.3±3.6, 7
	10	+3.1±1.7, 6*	+0.2±1.5, 6	+0.4±1.3, 6
11	5	+2.5±1.6, 12	+0.9±2.3, 12	+1.6±2.3, 12
	10	+3.0±0.9, 11*	+0.9±1.1, 12	+0.5±0.8, 10

*Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).*

In contrast, adaptation to +10 D lenses (myopic defocus) was not prevented by periods of normal visual stimulation (Table 3.4.5; Fig. 3.4.3, day 5; Fig. 3.4.5, day 10). Hyperopic shifts in refraction were always seen with the +10 D lens, although the magnitude of the hyperopia varied in proportion to the duration of lens wear per day. At day 5, +7.5±2.4 D, +6.0±1.4 D, +4.9±2.42 D, and +2.5±2.7 D of hyperopia were observed for

the 3, 6, 9 and 11 hr normal vision treatment groups respectively. Similarly, on day 10, a mean hyperopic shift of +5.8±2.2 D was produced even when the duration of lens wear was reduced by 3 hrs, i.e. 3 hrs of normal vision per day was given along with the refractive defocus. Similar hyperopic shifts, i.e. approximately 3 D, were observed for the 6, 9 and 11 hr treatment groups. Even 11 hours of normal vision per day, i.e. only 1 hr of lens wear, failed to entirely prevent the refractive adaptation to +10 D lenses. Plano-lens wear produced little variation in refraction when compared with the refraction of contralateral normal eyes (Table 3.4.5; Fig. 3.4.3, day 5; Fig. 3.4.5, day 10).

Ocular adaptation for refractive defocus and periods of normal vision

The changes in refraction produced by intermittent lens wear, like constant lens wear, were primarily due to alterations in VCD growth (Fig. 3.4.4, day 5; Fig. 3.4.6, day 10). For –10 D lens groups, i.e 3, 6, 9, 11 hr treatment groups, there was no significant refractive effect and also no change in VCD. In contrast, for the +10 D lens groups, the VCDs of treated eyes were significantly less than contralateral normal eyes for all treatment groups. Reductions in VCDs of 0.29±0.18 mm, 0.19±0.09 mm, 0.22±0.14 mm, 0.15±0.09 mm, and 0.11±0.08 mm were recorded for the 0, 3, 6, 9, and 11 hr treatment groups respectively, on day 5 and similar trends were observed on day 10 (0.16±0.12 mm, 0.15±0.14 mm, 0.05±0.13 mm, 0.10±0.08 mm, and 0.11±0.12 mm respectively). Differences in VCD between eyes were also highly correlated to refractive changes for both the +10 D and –10 D treatment groups (Fig. 3.4.8, day 5; Fig 3.4.9, day 10).

There was no obvious pattern to measured changes in ACD at either day 5 or 10 (Fig. 3.4.4, day 5; Fig. 3.4.6 day 10). At day 5, there was no correlation between interocular differences in ACD and refractive errors (Fig. 3.4.8), although at the later age these differences were correlated for the +10 D group (r = 0.884, $P < 0.05$; Fig 3.4.9). There was no effect of constant lens wear on ALT and similarly intermittent lens wear also had no effect on ALT (Fig. 3.4.4, day 5; Fig. 3.4.6, day 10).

Corneal power with refractive defocus and daily periods of normal vision

Lens wear had no significant effect on corneal curvature measured at day 5. However, corneal flattening, i.e. the cornea of the treated eyes was less powerful than normal, was evident at day 10 and increased as a function

of hours of lens wear per day (Fig. 3.4.7). This trend was observed for all lens types, irrespective of their sign, although it was most pronounced for the +10 D lens group. For the +10 D lens, 6 hrs of lens wear flattened the cornea by –0.7±2.3 D, 9 hrs by –2.6±2.8 D, and 12 hrs by –5.8±1.0 D.

In vitro measurements with refractive defocus and daily periods of normal vision

There were large variations in the external axial length results (Fig. 3.4.10). The trend was for eyes wearing positive spectacle lenses for more than 1 hour per day to have shorter axial lengths than their associated normal controls and for eyes wearing negative spectacle lenses to have longer external axial lengths, although the latter effect was only seen when the lens was worn for more than 6 hrs per day. For the plano lens treatment group, the difference was not statistically significant although there was a large spread in the individual data.

Equatorial diameter did not vary significantly with lens power, although there was a slight trend towards increased equatorial diameter for eyes wearing lenses. This effect was limited to eyes wearing lenses for more than 6 hrs per day and was not dependent on lens power. Wet eye weight was not consistently or significantly affected by lens wear.

Morning cf evening refractive defocus

For the 1 hr lens wear treatment groups, there were significant differences ($P < 0.05$, MWUT, +10 D and –10 D) in the magnitude of refractive shifts depending on whether the period of lens wear was given in the morning (am) or the afternoon (pm) for both the +10 D and –10 D treatment groups at day 5 (Fig. 3.4.11). Greater refractive shifts in response to lens wear occurred for the am +10 D lens group and pm –10 D lens group . Vitreal chamber growth reflected these changes, with greatest effects being observed with the am +10 D lens group ($P < 0.05$, MWUT) and pm –10 D lens group. The differences between related am and pm groups were also statistically significant in the case of the +10 D group. These differences between groups were not evident in the am and pm data at day 10. Although lens wear was also split into am and pm groups for the other lens wearing paradigms, the numbers were too low for statistical analysis.

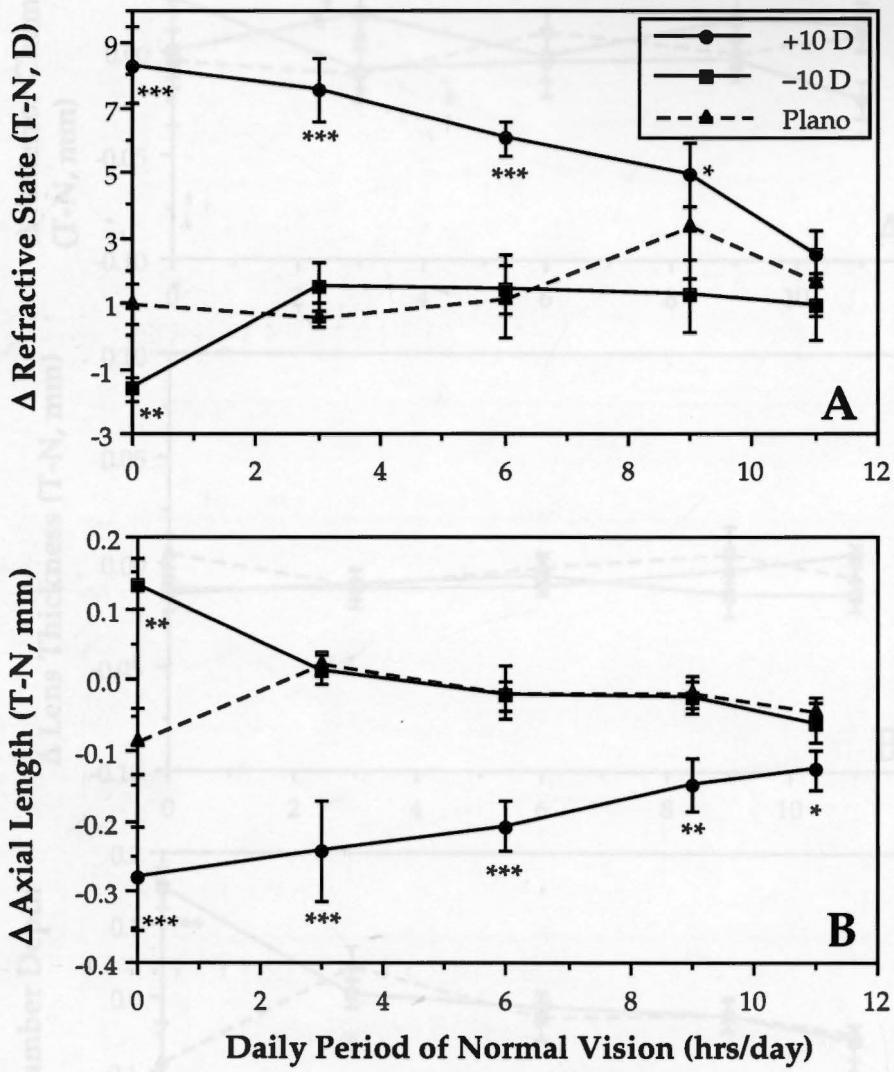


Figure 3.4.3. Differences (mean ± SE), at day 5, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

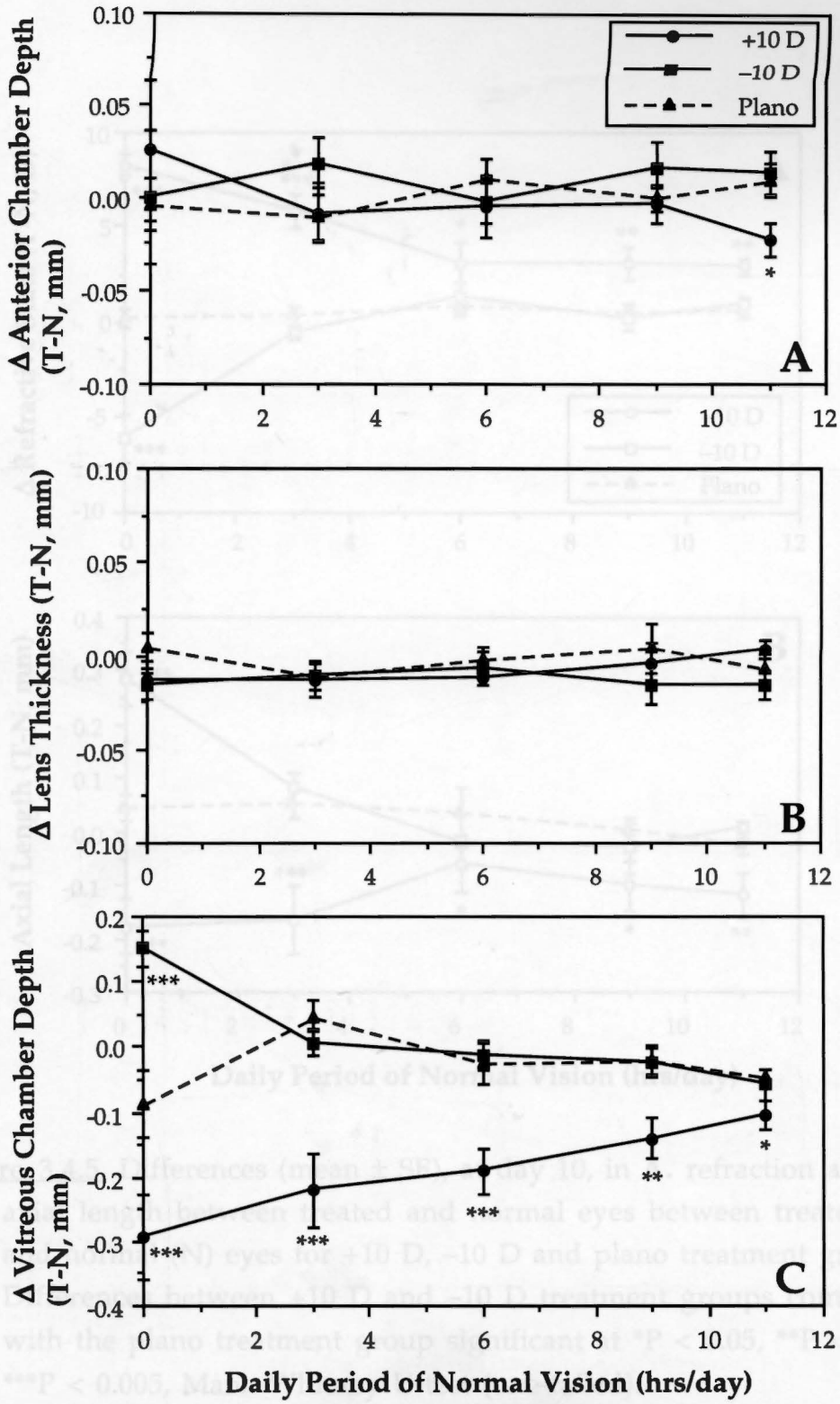


Figure 3.4.4. Differences (mean ± SE), at day 5, in **A.** anterior chamber depth **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

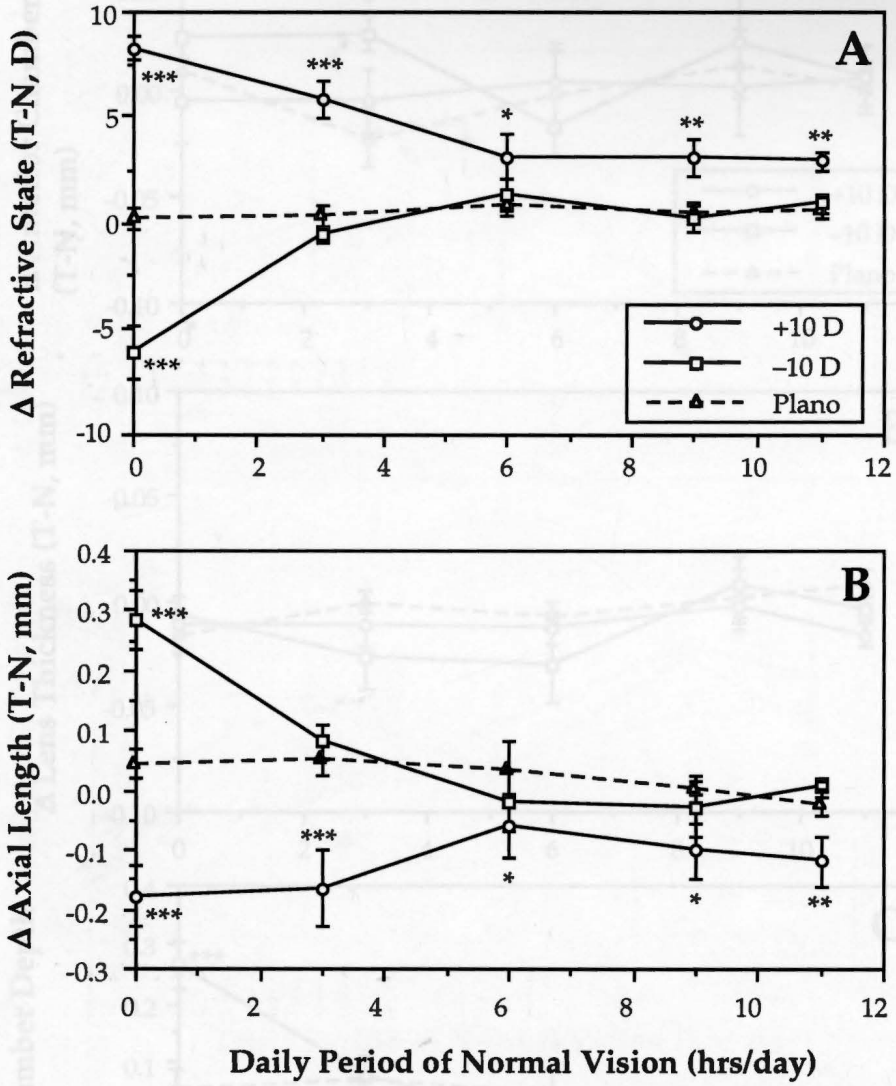


Figure 3.4.5. Differences (mean ± SE), at day 10, in **A.** refraction and **B.** axial length between treated and normal eyes between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

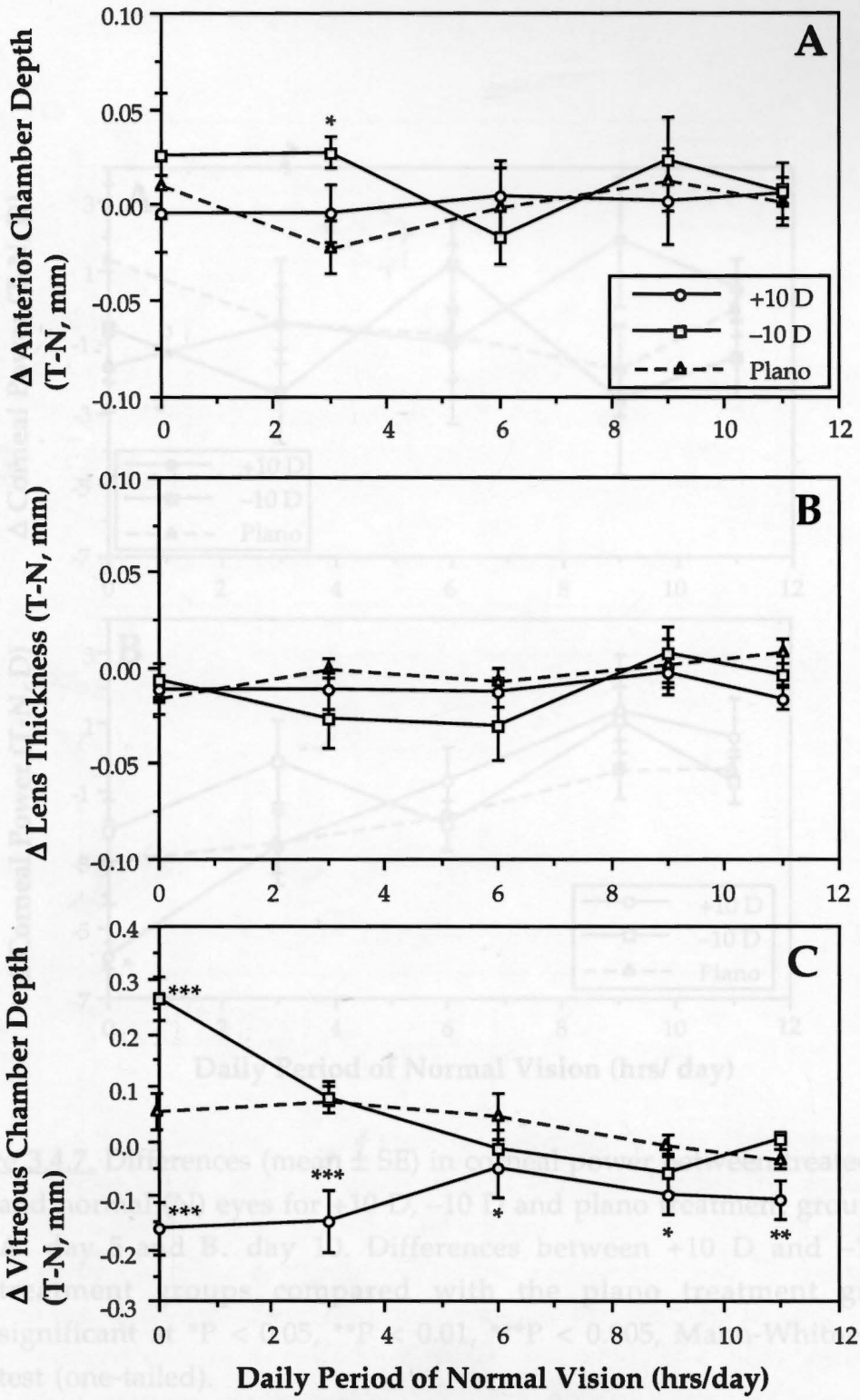


Figure 3.4.6. Differences (mean ± SE), at day 10, in **A.** anterior chamber depth **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

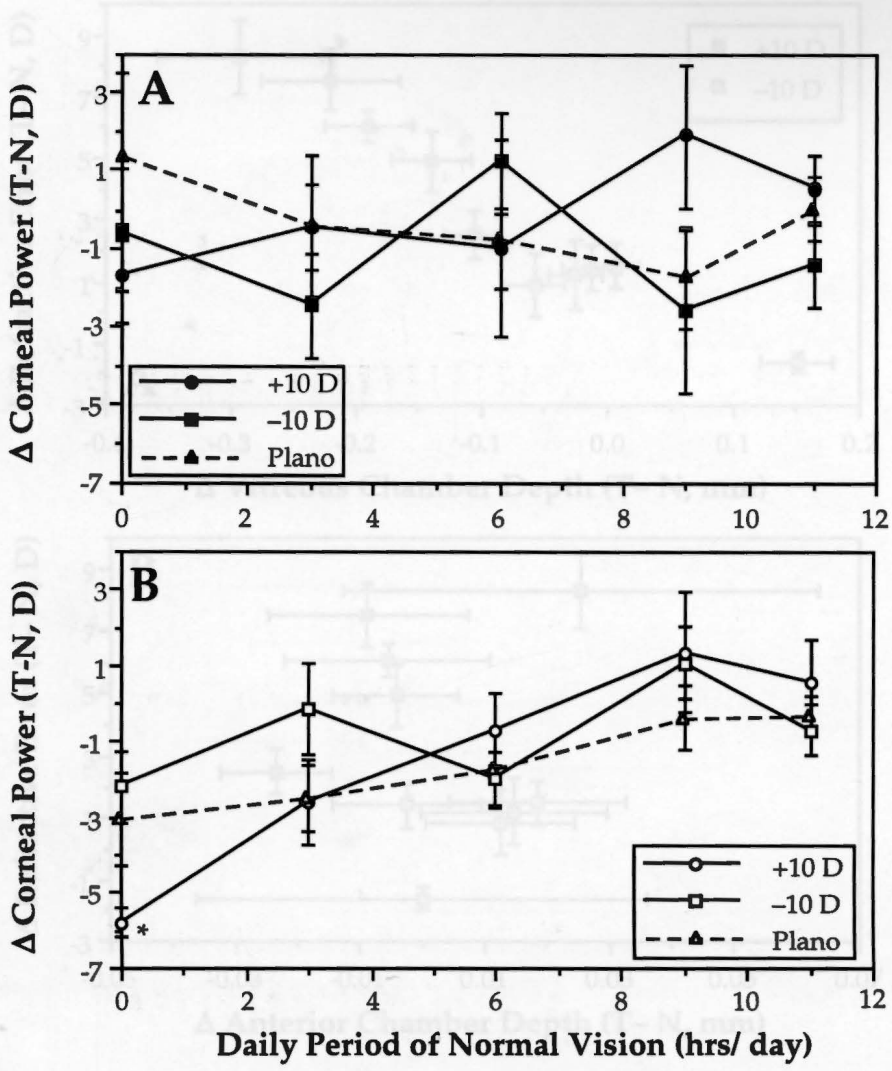


Figure 3.4.7. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups at **A.** day 5 and **B.** day 10. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

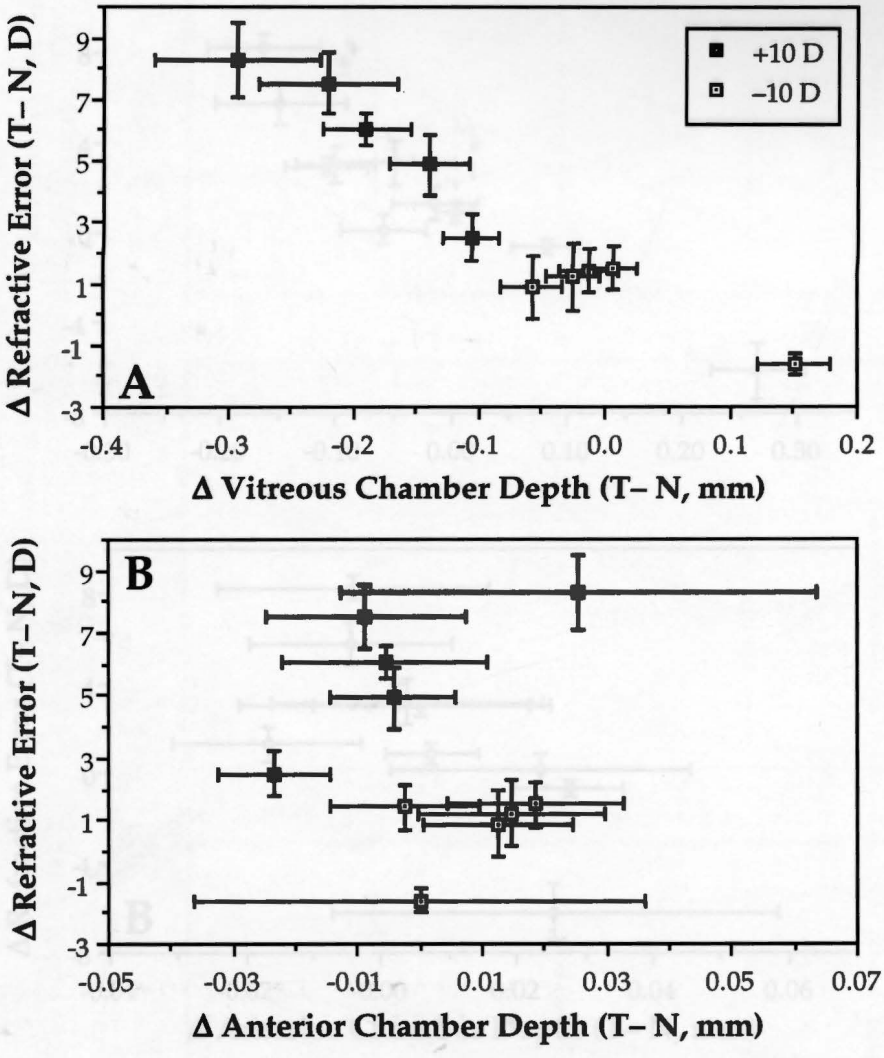


Figure 3.4.8. Relationship, at day 5, between mean differences in refractive error (ΔRE) between treated (T) and normal (N) eyes and mean differences in **A.** vitreous chamber depth (ΔVCD) and **B.** anterior chamber depth (ΔACD), for +10 D and −10 D treatment groups. ΔRE and ΔVCD were correlated for the +10 D and −10 D treatment groups (r = 0.950, P < 0.01; r = 0.891, P < 0.05), ΔRE and ΔACD were not correlated.

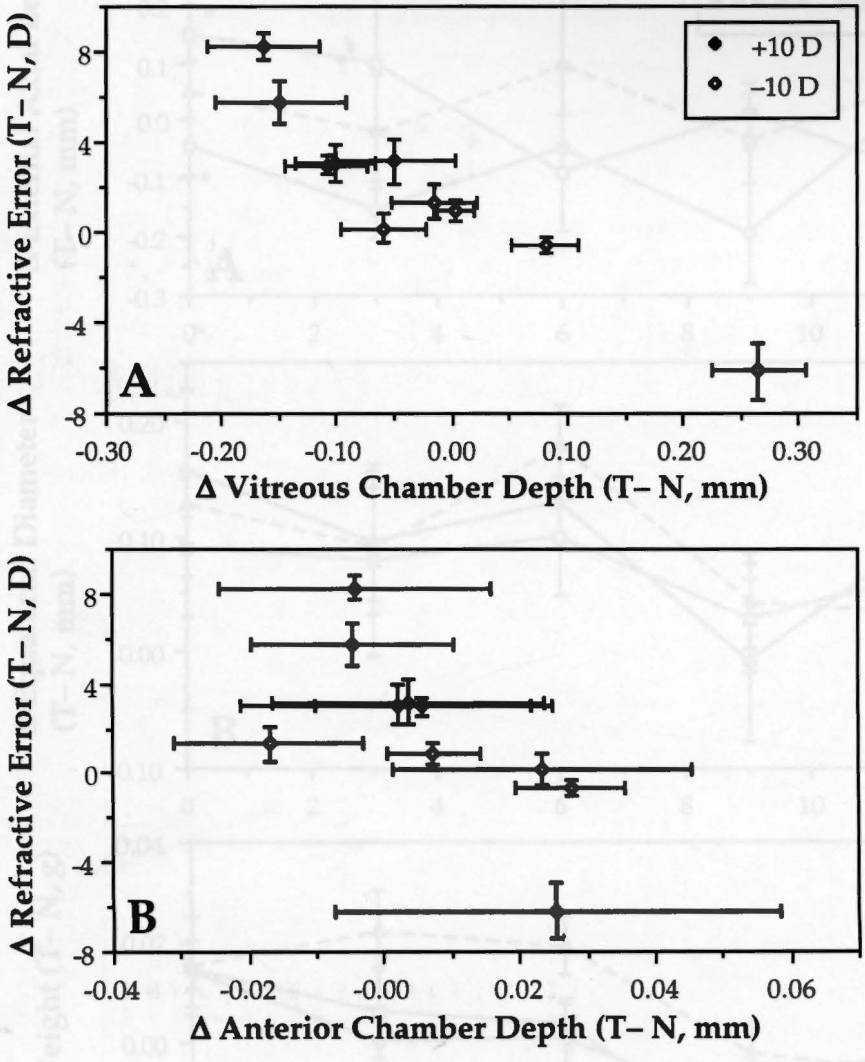


Figure 3.4.9. Relationship, at day 10, between mean differences in refractive error (ΔRE) between treated (T) and normal (N) eyes and mean differences in **A.** vitreous chamber depth (ΔVCD) and **B.** anterior chamber depth (ΔACD), for +10 D and −10 D treatment groups. ΔRE and ΔVCD were correlated for the +10 D and −10 D treatment groups (r = 0.826, $P < 0.05$; r = 0.943, $P < 0.02$), ΔRE and ΔACD were correlated for the +10 D group only (r = 0.884, $P < 0.05$).

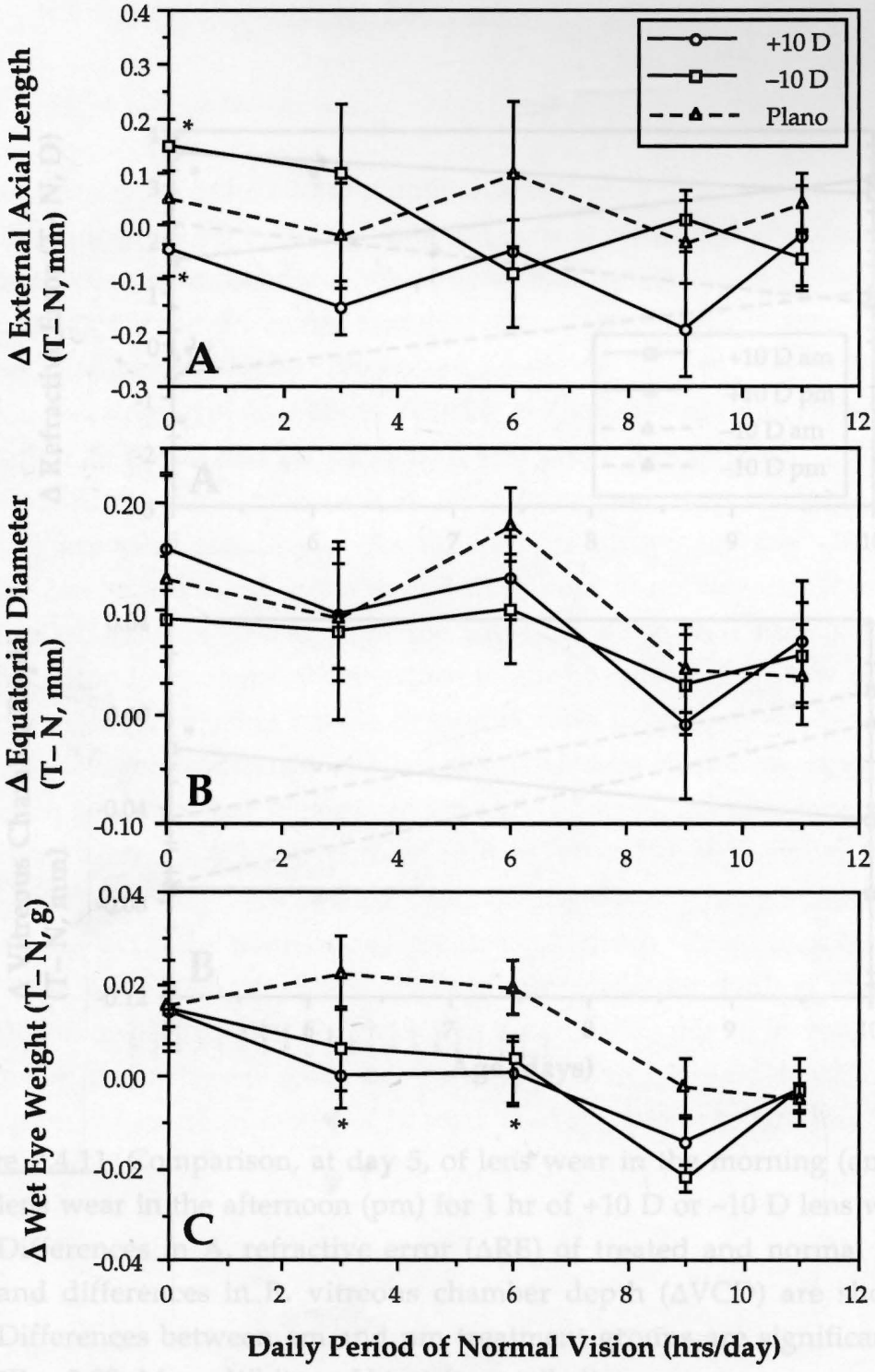


Figure 3.4.10. Differences (mean ± SE) in **A.** external axial length, **B.** equatorial diameter and **C.** wet eye weight between treated (T) and normal (N) eyes for +10 D, –10 D and plano treatment groups, at day 10. Differences between +10 D and –10 D treatment groups compared with the plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

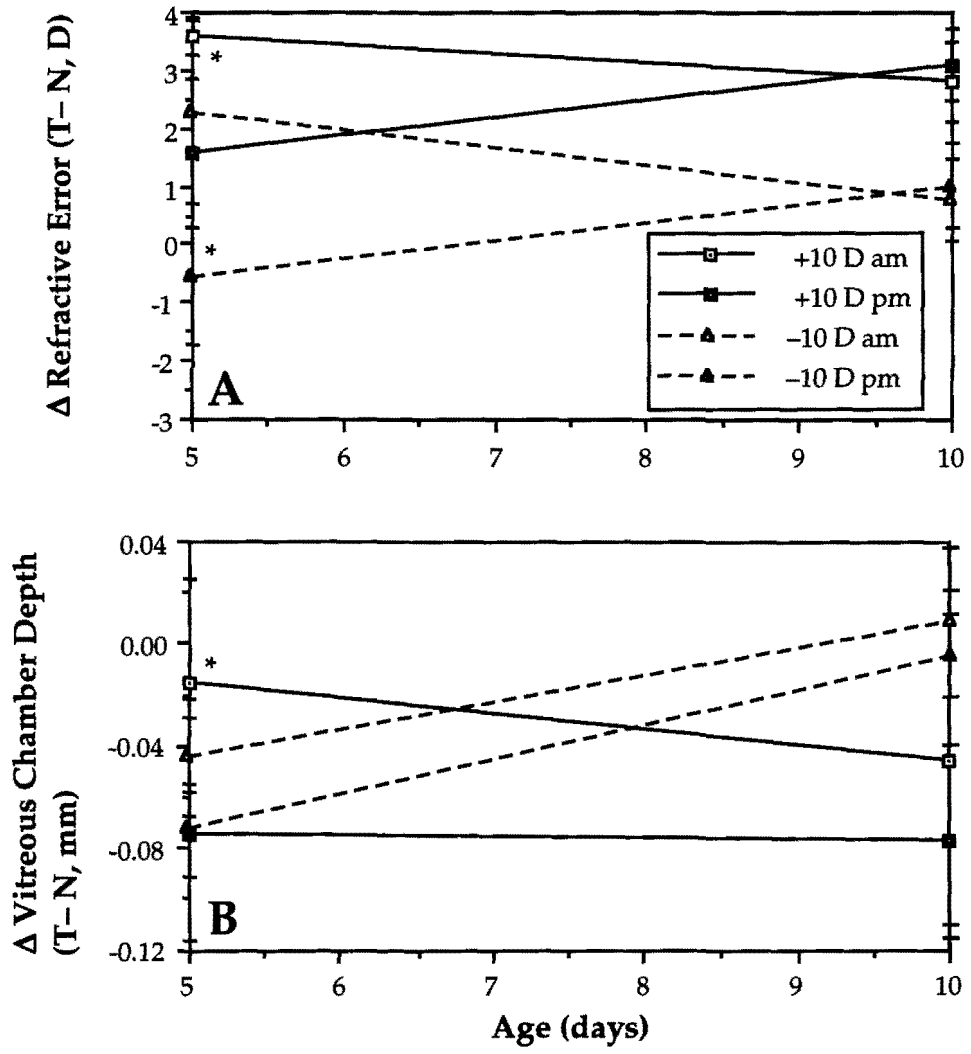


Figure 3.4.11. Comparison, at day 5, of lens wear in the morning (am) to lens wear in the afternoon (pm) for 1 hr of +10 D or –10 D lens wear. Differences in **A.** refractive error (ΔRE) of treated and normal eyes and differences in **B.** vitreous chamber depth (ΔVCD) are shown. Differences between am and pm treatment groups are significant at *$P < 0.05$, Mann-Whitney U-test (two-tailed).

3.4.4. Discussion

Constant refractive defocus

Constant lens wear induced adaptational ocular growth responses which were determined by both the sign and magnitude of the induced defocus. Negative lens wear resulted in myopia and positive lens wear in hyperopia and these refractive changes were affected by adjustments to vitreous chamber, with positive lenses inhibiting growth and negative lenses increasing growth. These results are consistent with those of Schaeffel *et al.* (1988) and Irving *et al.* (1991) and support the hypothesis that the chick eye can determine both the sign and amount of defocus and alter eye growth accordingly. As the rate of growth of the vitreous chamber can be increased or decreased from normal implies that it is the detection of defocus, rather than the physical sense that the vitreous chamber is too long or too short (which might be corrected by any shape sensitive mechanism), that results in modification to eye growth.

Adaptation to constantly applied positive lenses was more rapid and more complete than that to negative lenses. At 5 days, positive lens wear had caused a mean +8.3 D hyperopic shift in refraction, in comparison to a myopic shift of only –1.6 D with negative lens wear. Why is adaptation to constant –10 D lens wear (hyperopic defocus) much slower than that to +10 D lens wear (myopic defocus)? Results of form-deprivation studies rule out the possibility that the chick eye is simply unable to increase the growth of the vitreous chamber to a sufficiently high rate; form deprivation can cause in excess of 10 to 12 D of myopia to be produced by day 5 (section 3.1).

Refractive defocus with daily periods of normal vision

Hyperopic shifts and slowed axial eye growth occurred for the +10 D treatment group, even when periods of normal vision were introduced. This result is in contrast to the –10 D lens data where myopic shifts in refraction and increased ocular growth only occurred if lens wear was not interrupted. This result is shown schematically in Fig 3.4.12. The results indicate that the "control" system of the young chick will "decrease its growth" with minimal stimulation in response to positive lenses; some adaptation to plus lenses occurs with as little as 1 hr of wear per day.

However although the control system is presumably the same, the chick eye is unable to increase growth in response to a negative lens unless the lens is worn for a significant period of the day, indeed almost continuously. In this latter respect, negative lenses appear to mimic the effect of form deprivation in that both effects are very sensitive to interruption of treatment with periods of normal vision.

For 3, 6 and 9 hr treatment groups greater adaptation to +10 D lens wear (myopic defocus) occurred at day 5, the early measurement point, than at day 10. The reason for this may be the increased lens loss during the period of wear between day 5 and 10. Although chicks were monitored at very regular intervals, short periods without lenses could not be totally avoided in older birds which were more adept at removing their lenses.

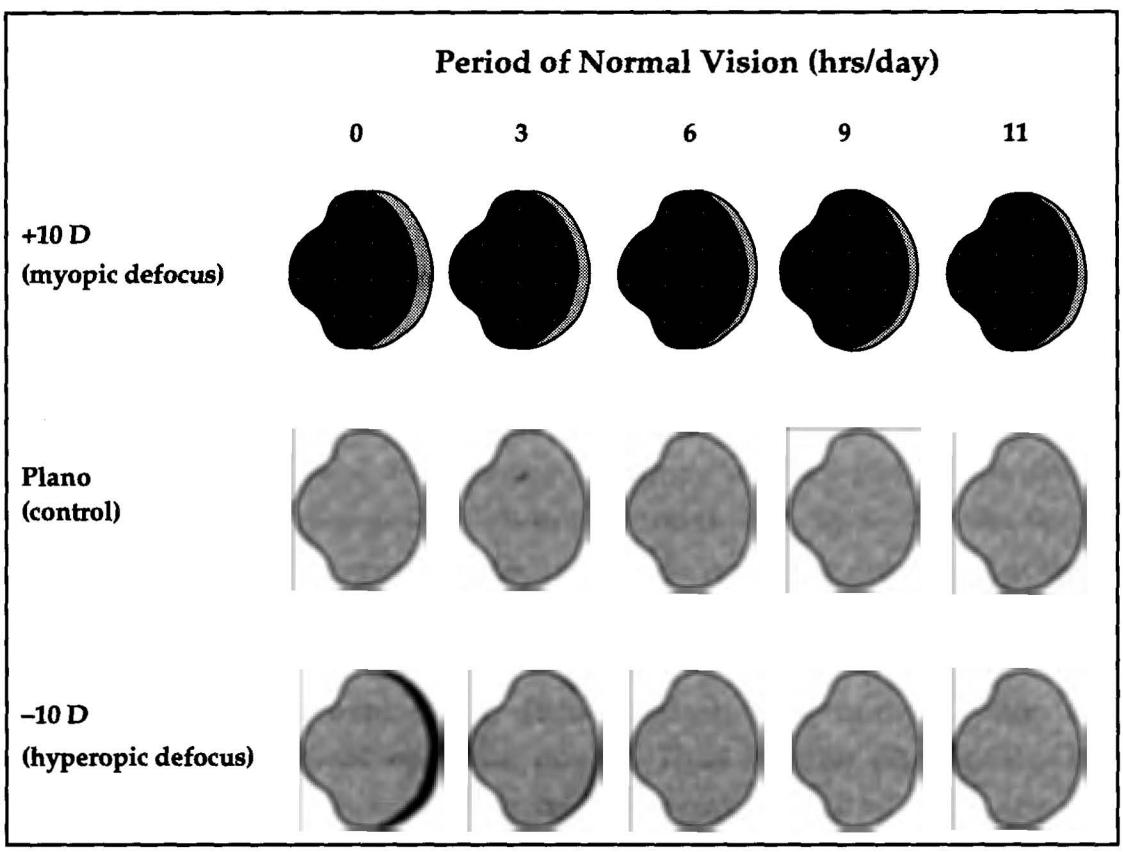


Figure 3.4.12. Schematic representation of the ocular changes that occur with periods of both refractive defocus and normal vision. The darkly shaded eyes represent treated eyes and the lightly shaded the normal eyes. Changes are primarily due to increased or decreased vitreous chamber depth.

Reason for nonlinearity

Adaptation to negative lenses was much less than that to positive lenses, more affected by normal vision and also much slower. Nonlinearity of adaptation was also noted by Schaeffel *et al.* (1988) who observed less refractive responses for negative compared with positive lenses and by Irving *et al.* (1991) who observed the slower adaptation for negative lenses. A contributing factor to this differential response to negative and positive lenses may be that the positive lenses provide a more constant level of retinal defocus compared with negative lenses; with positive lenses, the retinal image will be constantly blurred during distance viewing, while with negative lenses defocus may be overcome through accommodation. The drug-induced amplitude of accommodation of the chick is particularly high at young ages, i.e. 21 D at day 2 decreasing to 11 D at day 10 (section 4.2) and these data are consistent with observations of 15 D of accommodation under natural conditions (Schaeffel *et al.*, 1986). The decrease in accommodative amplitude with age might explain the greater response of negative lenses at day 10 when it seems unlikely that chicks would be able to sustain sufficient accommodation to maintain clear focus, even of distant objects; near objects would be more affected.

An alternative explanation for the differences in response to positive and negative lenses relates to differences in the physiological basis of observed changes in growth. It is now recognized that choroidal swelling contributes to the decrease in vitreous chamber depth observed with positive lenses and that this process may be very fast (Wallman *et al.*, 1992). While changes in choroidal thickness also contribute to vitreous chamber changes induced by negative lenses enhanced scleral growth is largely responsible for vitreous chamber expansion and this process, which involves "real growth", is likely to be much slower. This can also be used to explain the differential effect of normal vision on adaptation to lenses of opposite power. Thus for the plus lens case, adaptation to the lenses may occur relatively quickly and when lenses are removed a hyperopic refractive error is likely to be present. As this then requires increased growth or choroidal thinning, recovery from the hyperopia may be slow and residual hyperopia observed, the degree of residual hyperopia thus may reflect the differential speed and duration of different types of growth mechanisms. In contrast, for the minus lenses, adaptation to the lens is likely to be slow and when the lenses are

removed only a low myopic refractive error is likely to present. As emmetropizing changes occur very rapidly for this type of error, no residual myopia is seen. It would be predicted that if the period of normal vision were reduced further, a point would eventually be reached where some residual myopia would be observed.

As an alternative explanation for the differential rates of adaptation, the following model may also be plausible. As refractive recovery from form-deprivation myopia occurs extremely quickly (section 2.1), this may indicate the presence of a nonvisual growth mechanism which drives the shape of the eye towards normal proportions. When growth away from normal proportions is required for emmetropization, growth may be much slower due to this opposing signal. This theory could explain the differential effects of positive and negative lenses, where for positive lenses a halting of growth is required for emmetropization while for negative lenses increased growth away from normal proportions is required.

Wildsoet and Wallman (1992) have also recently shown that ocular compensation to spectacle lenses is reduced for negative but not for positive lenses following optic nerve section. Optic nerve section, in addition to disrupting accommodation feedback loops, causes the degeneration of retinal ganglion cells (Ehrlich, 1981; Wildsoet and Pettigrew, 1989). Thus, the greater effect of optic nerve section on the response to negative lenses may indicate that slightly different retinal processing is required for analysis of myopic and hyperopic defocus, the disruption of retinal processing thus having a greater effect for emmetropization to negative lenses. Alternately, the difference may reflect other physiological changes resulting from optic nerve section.

Comparison to predictions

To study the relative contributions of the periods of lens wear and normal vision to the resultant refractive error, predictions about the expected refractive error were made on the basis of hours of experience using a model which gives equal weighting to lens wear and normal vision (Fig. 3.4.13). The underlying assumption for this model was that if the periods of lens wear and normal vision were equivalent in terms of effects on eye growth, then a lens worn for half the time would produce half the refractive change compared with a lens worn constantly. Full

adaptation with constant wear was also assumed. Thus it was predicted that constant lens wear should produce a 10 D refractive change, with the introduction of 3 hrs of normal vision reducing refractive adaptation to 9/12 of this value, i.e. 7.5 D. Likewise, with 6 hrs, i.e. half normal vision and half lens wear, eyes should show 50% adaptation, i.e. 5 D and so on to give predicted refractions of 2.5 D with 9 hrs normal vision and 0.8 D with 11 hrs normal vision. This model does not take into account possible differences in the speed of adaptation.

For the case of myopic defocus, a large amount of hyperopia resulted from constant defocus. The amount of hyperopia progressively declines with increasing periods of normal vision and shows relatively close correspondence to predicted values. In contrast, with hyperopic defocus, changes were always much lower than predicted. This reflected the only low myopic refractive errors seen with constant lens wear and the negligible effects on refraction when normal vision was introduced.

Chicks did not adapt to hyperopic defocus even when treatment was applied for three quarters of the day. This result might indicate that the chicks were able to maintain an adequate accommodation to eliminate blur over this period, preferring a high accommodative demand, for 9 hrs out of 12 to distance blur, for 3 hrs out of 12, which would have been the case if compensation had occurred. This interpretation, if valid, also questions the theory that high levels of accommodation cause myopia given that a presumed 10 D of extra accommodative activity for 3/4 of the day had no effect on refractive error. Irving et al. (1991) suggests that chicks only intermittently accommodate through lenses. Issues relating to accommodation and the response to negative lenses have been further investigated in experiments reported in Chapter 4.

In summary, the results for the positive lenses support the hypothesis that for eye growth mechanisms equal weighting is given to periods of both lens wear and normal vision, while the results for the negative lenses support the prediction, that myopic defocus is equally sensitive to periods of normal vision irrespective of the manner in which it is generated, i.e. whether from form-deprivation or negative lens wear.

Competing growth signals

Another way of looking at the difference between positive and negative lenses, is in terms of eye growth signals (Fig. 3.4.14). As constant +10 D lens wear, i.e. constant myopic defocus, produced shorter than normal eyes these lenses may be considered to be activating a "stop" signal that slowed eye growth. Conversely, constant –10 D lens wear, i.e. hyperopic defocus, resulted in longer eyes than normal, and thus can be considered linked to a "go" signal which increases ocular growth. During normal growth similar signals are generated which depend on the eye's refractive error.

When periods of both lens wear and normal vision are intermixed, competing eye growth signals are experienced. For the +10 D (myopic defocus) treatment group, eyes receive both a "stop" signal, when wearing positive lenses and either a "go" signal or "normal" signal when no lens is in place depending on whether compensation occurred for the +10 D lens or whether the eye is emmetropic. Here, both signals seem to have significant weighting in determining eye growth with the result being a "stop" signal which is progressively dampened with increasing exposure to normal vision. In contrast, for –10 D lens wear (hyperopic defocus), eyes receives both a "go" signal when wearing minus lenses and a "stop" signal or "normal" signal when no lens is in place, again depending on whether adaptation to the lens has occurred. In this case, results suggest that the "stop" or "normal" signal has a greater weighting than the "go" signal as there was no change in refraction when periods of normal vision are introduced. Thus, it seems that the "go" signal for increased eye growth is very easily switched off by the "normal" signal and this contrasts with the "stop" signal which appears much stronger. If some adaptation to negative lenses did occur during the period of lens wear, then a myopic defocus error would be present at the time of presentation of the period of normal vision. This has analogies to results in the first part of this chapter which show that prevention of form-deprivation myopia occurs with extremely short periods of normal vision. Presumably similar eye growth signals are experienced during the period of normal vision and thus the similarity in effect is explained.

The greater effectiveness of the "stop" signal on eye growth may indicate that the visual system has an in-built safeguard against growing too long and becoming permanently myopic. This would seem

appropriate as the chick eye has a limited time frame over which true recovery from myopia, i.e. by adjustments of sclera growth, can occur. It may also be physiologically easier to increase eye growth if still hyperopic at a critical time point in eye development, rather than to halt eye growth if myopic. Alternatively a slightly hyperopic refractive error may affect function less than a myopic refractive error due to the actions of accommodation and this also argues in favour of an eye growth system that is highly sensitive to myopic defocus.

Normal growth signals

The results which show that "stop" and "go" growth signals can be induced by artificial means can be extrapolated to the normal situation. It would appear that there are two mechanisms of eye growth. The first is related to overall body growth; as the chick develops all body parts, including the eyes show some proportional growth. The second system acts to modulate the refractive state during this growth period and by adjusting the relative growth rates of the anterior and posterior segments to ensure emmetropia is maintained, i.e. it fine tunes eye growth. In this way if hyperopic defocus is detected, a "go" signal is generated and the growth of the vitreous chamber increases relative to normal and, if myopic defocus is detected, a "stop" signal is generated and the growth of the vitreous chamber slows relative to normal. The "stop" signal is very potent such that if myopia is detected, the growth of the vitreous chamber is slowed immediately. The eye is more tolerant to hyperopic defocus and increased growth relative to normal only occurs if the defocus is experienced continually. Once body growth ceases, eye growth presumably ceases as well; by monitoring the refractive state during development this enables the refractive state to be fine tuned and prevents the development of refractive errors during a time of active growth when they are presumably most likely to occur.

While it is suggested that growth is controlled within the eye (Wildsoet and Pettigrew, 1987; Wallman *et al.*, 1987), the cells which detect the defocussed image and the chain of events that link the defocus signals to ocular growth remain unknown. It is suspected that retinal cells, most likely amacrine cells (Wallman, 1991), are involved in this process. The retinal transmitter dopamine has also been implicated; dopamine levels are reduced in form deprived eyes and treatment with

dopamine agonists such as apomorphine decreases form-deprivation myopia (Stone *et al.*, 1989). Retinal labelling for GABA, glycine, glutamate and taurine appears to be unaffected by form deprivation, suggesting that these putative neurotransmitters are not involved in the eye growth regulation (Appendix III), although immunolabelling techniques are not as sensitive as high-pressure liquid-chromatography (HPLC) assays used in the dopamine study. One substance or multiple substances may regulate eye growth with the involvement of a signal cascade. Release or inhibition of certain retinal transmitters may initiate the cascade, with growth factors such as bFGF at the end acting on the sclera and/or choroid to alter growth.

Comparison to lens wear in other animals

Not all animals respond to refractive defocus in the same way as the chick. Nathan *et al.* (1984) showed using hard contact lenses, that it was the magnitude rather than the direction of defocus which determined the cat's response to defocus; myopia was always produced. Thus while large hyperopic refractive errors were produced by myopic defocus in the chick, myopic refractive errors occurred in the cat. The refractive error changes were also not dramatic; the largest myopic refractive error they observed was –2.5 D. Their observation of myopia in response to positive lenses also provides another perspective on the role of accommodation in refractive error control; in this example, that the lenses should relax rather than stimulate accommodation suggests that accommodative activity is not a necessary prerequisite for the development of myopia as sometimes argued for humans.

Relationship to human data

The chick data indicate that both constant and intermittent positive spectacle lens wear results in adaptation which varies in magnitude with the duration of wear, whereas adaptation to negative powered spectacles only occurs if the lenses are worn constantly. Reports of adaptation to spectacle lenses in humans are conflicting, with Atkinson *et al.* (1987) reporting no effect of positive lens wear on the refractive development of human infants and Dobson *et al.* (1986) reporting poorer emmetropization, i.e. less reduction in hyperopic refractive errors, for

strabismic infants wearing positive lenses compared with those not given spectacles. The existence of spectacle lens adaptation in humans is thus still subject to debate, although on the basis of the chick data described here, care should be taken not to "over correct" individuals if emmetropization is to follow its normal course.

3.4.5. Conclusion

Myopia only develops if hyperopic defocus is applied continuously; in contrast hyperopic changes with myopic defocus are seen even when periods of normal vision are introduced. The similarity of the effect of normal vision on form-deprivation and lens-induced myopia suggest that similar emmetropizing growth signals are experienced during the period of normal vision regardless of the method of myopia production.

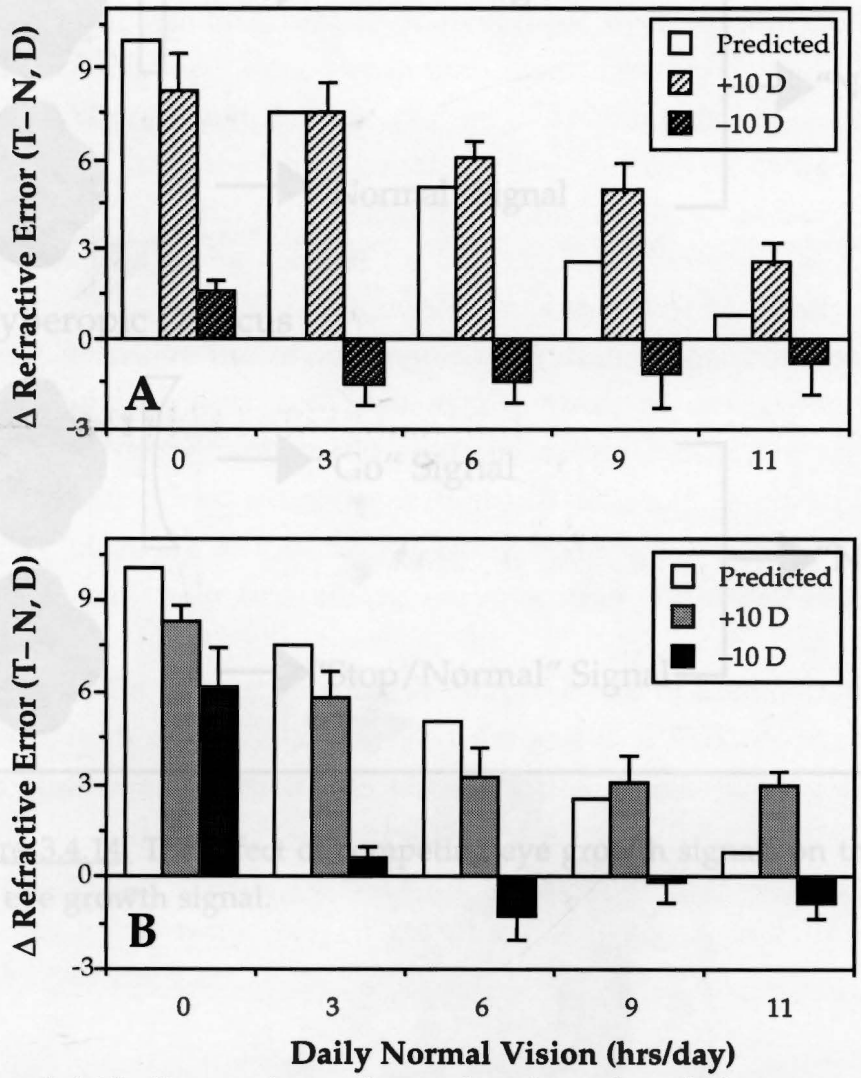


Figure 3.4.13. Comparison of actual and predicted changes in refractive error for +10 D and –10 D treatment groups at **A.** day 5 and **B.** day 10.

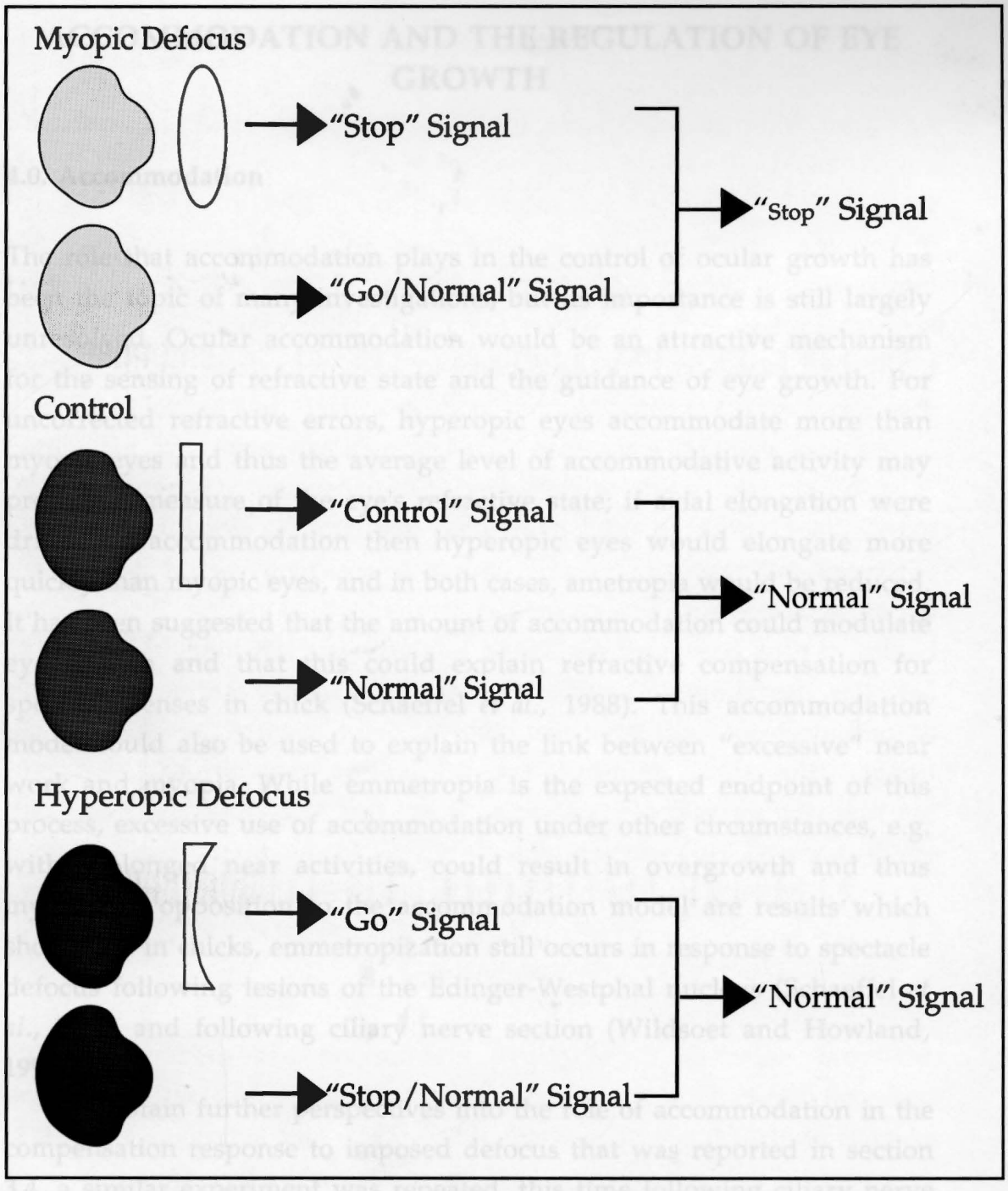


Figure 3.4.14. The effect of competing eye growth signals on the resultant eye growth signal.

CHAPTER 4

ACCOMMODATION AND THE REGULATION OF EYE GROWTH

4.0. Accommodation

The role that accommodation plays in the control of ocular growth has been the topic of many investigations, but its importance is still largely unresolved. Ocular accommodation would be an attractive mechanism for the sensing of refractive state and the guidance of eye growth. For uncorrected refractive errors, hyperopic eyes accommodate more than myopic eyes and thus the average level of accommodative activity may provide a measure of the eye's refractive state; if axial elongation were driven by accommodation then hyperopic eyes would elongate more quickly than myopic eyes, and in both cases, ametropia would be reduced. It has been suggested that the amount of accommodation could modulate eye growth and that this could explain refractive compensation for spectacle lenses in chick (Schaeffel *et al.*, 1988). This accommodation model could also be used to explain the link between "excessive" near work and myopia. While emmetropia is the expected endpoint of this process, excessive use of accommodation under other circumstances, e.g. with prolonged near activities, could result in overgrowth and thus myopia. In opposition to the accommodation model are results which show, that in chicks, emmetropization still occurs in response to spectacle defocus following lesions of the Edinger-Westphal nucleus (Schaeffel *et al.*, 1990) and following ciliary nerve section (Wildsoet and Howland, 1991).

To obtain further perspectives into the role of accommodation in the compensation response to imposed defocus that was reported in section 3.4, a similar experiment was repeated, this time following ciliary nerve section of treated eyes (section 4.1). The effect of altering the gain of the accommodative system, i.e. reducing the magnitude of accommodation by preventing the refractive effects of corneal accommodation, was also studied (section 4.2).

4.1. Accommodation and Refractive Adaptation

4.1.0. Summary

The effect of ciliary nerve section (CNS) and limited duration refractive defocus was investigated. CNS was performed on day 2; chicks wore a spectacle lens (+10 D, –10 D, or plano) from day 7 to day 11. Lenses were worn either constantly (0) or lens wear was interrupted with a period of normal vision (3, 6, or 9 hrs per 12 hr day). CNS caused mydriasis and eliminated accommodation. The combination of CNS and plano lens wear resulted in deeper anterior chambers, thinner lenses and slight vitreous chamber and axial elongation, but no significant refractive effect. Significant hyperopia was observed with constant +10 D lens wear (+10.6 D) and conversely, constant –10 D lens wear produced myopia (–6.3 D). Only slight myopia (–2.5 D) was seen when 3 hrs of normal vision were introduced; further increases in normal vision completely prevented the development of myopia. By contrast, hyperopia was always seen with +10 D lenses, although the magnitude of hyperopia decreased with increased duration of normal vision. In all cases, refractive changes largely reflected altered vitreous chamber depth, myopia and hyperopia corresponding to longer and shorter than normal vitreous chambers. The results suggest that there are both "go" and "stop" signals for ocular growth that are activated by hyperopic (–10 D) and myopic defocus (+10 D) respectively and that these signals are not linked to accommodative activity.

4.1.1. Introduction

Currently, there are no models available to describe how the accommodative tonus might influence eye growth in chickens. Schaeffel *et al.* have suggested that one possible mechanism could include changes in intraocular pressure occurring as a consequence of changes in accommodative tonus (Schaeffel *et al.*, 1988; Schaeffel and Howland, 1991). However, experiments in chicks show that emmetropization still occurs in response to refractive defocus following lesions of the Edinger-Westphal nucleus (Schaeffel *et al.*, 1990) and following ciliary nerve section (Wildsoet and Howland, 1991); that emmetropization occurs in the absence of accommodation suggests that accommodation is not essential for emmetropization. The fact that only the deprived section of

the eye elongates in response to partial occluders, also argues against accommodation having a major role in eye growth (Wallman *et al.*, 1987; Miles and Wallman, 1990). It should be noted, however, that in the Edinger-Westphal nucleus lesioning study, slightly hyperopic refractions were present before lens application; the chick eye did not have to change its refractive power to appear to have adapted to the positive spectacle lenses. Similarly, while emmetropization still occurred when accommodation was blocked following ciliary nerve section, end refractions tended to be slightly more hyperopic than those of control eyes (Wildsoet and Howland, 1991).

In the previous section (3.4), it was shown that the eye responds differently to short periods of negative and positive spectacle lens wear, with adaptation to positive lenses varying in proportion to the duration of lens wear and adaptation to negative lenses occurring only for constant wear. One hypothesis put forward to explain these results implicated accommodation; arguments were based on the premise that with short periods of negative lens wear, the retinal image should appear clear due to accommodation, while for positive lenses, the retinal image would appear blurred for distance viewing. If retinal blur serves as a stimulus for the emmetropization mechanism then positive lenses should elicit a greater "response". This hypothesis was tested in the study outlined below by eliminating accommodation using ciliary nerve section. It was predicted that with the removal of accommodation the response to negative lens wear should be more similar to the response to positive lens wear, with some refractive changes occurring with intermittent negative lens wear.

4.1.2. Methods

Animals

Male, White Leghorn-New Hampshire crossbreed chicks were obtained from a local hatchery on the day of hatching. They were raised in temperature controlled enclosures with food and water provided *ad libitum*. Chicks were exposed to a 12 hr light/ 12 hr dark diurnal light cycle, with lights on at 7 am and off at 7 pm. A light intensity of 250 lux at the level of the food trough was provided by overhead fluorescent lights.

Ciliary nerve section

Ciliary nerve section (CNS) was performed on day 2 on the left eye of chicks anaesthetized with halothane. The posterior orbit was exposed via a small incision, 2 to 3 mm, made through the lower lid and the extraocular muscle cone split apart with forceps to expose the nerve. The nerve was then hooked and cut on the bulbar side of the ciliary ganglion. Two sutures were used to close the small incision; a prophylactic antibiotic was applied and the wound sealed with superglue (see Appendix I).

To determine the success or otherwise of the surgery: i) pupil light responses were tested on days 3, 6, 10 and 11, and ii) on days 6 and 10 infrared-video-photoretinoscopy (Schaeffel and Howland, 1987) was performed on awake chicks, during which they were encouraged to look at and clear near targets, i.e. accommodate over negative spectacle lenses (–4 D and –8 D). CNS eliminates accommodation and results in a widely dilated, unresponsive pupil. No chicks recovered either pupil or accommodative function over the experimental period (see Appendix I).

Lens wearing protocol

Chicks were reared with either a +10 D (myopic defocus), –10 D (hyperopic defocus), or plano (zero powered) spectacle lens (Table 4.1.1). Some chicks wore the lenses all day long and others wore the lenses for only part of the day. The +10 D and –10 D lenses were worn for either 3, 6, 9 or 12 hrs (constant wear) per 12 hr day, i.e. chicks experienced normal vision for either 9, 6, 3 or 0 (constant wear) hrs/day; the plano lens was applied constantly. All groups, except the plano group (n = 9), comprised 7 chicks. The period of lens wear was not split across the day; instead the period of normal vision was given in one complete block of time, always at the beginning (morning) or at the end (evening) of the light cycle.

The CNS surgery resulted in slight lid inactivity. To ensure that lid function had fully returned in all birds, lens treatment was delayed until day 7. Any chicks whose lid action showed no signs of recovery by day 4 were excluded from the study; 13 chicks were excluded for this reason. In addition, a control experiment was run where chicks wore lenses (–10 D, n = 7) from day 7 to 11, but did not undergo CNS.

The spectacle lenses used were of similar design to those used previously, i.e. 12 mm diameter PMMA contact lenses, with large optic

zones (10.5 mm to 11.5 mm) and 8.0 mm back optic radii. The lenses were applied to the chick's eyes by means of a velcro ring, one side being glued to the feathers surrounding the eye of the chick and the other to the lens. Chicks were checked 5 to 6 times per day, to reattach lost lenses and replace dusty/dirty lenses.

Table 4.1.1. Powers of spectacle lenses used and daily period of lens wear.

Spectacle lens	Defocus	Surgery	Period of lens wear (hrs/day)			
+10 D	Myopic	CNS	3 (9)	6 (6)	9 (3)	12 (0)
–10 D	Hyperopic	CNS	3 (9)	6 (6)	9 (3)	12 (0)
Plano	Control	CNS	–	–	–	12 (0)

Duration of daily normal vision bracketed.

Measurements

Chicks wore the spectacle lenses from days 7 to 11 after which time ocular measurements were performed. Chicks were anaesthetized using halothane, and retinoscopy (non-cycloplegic) and A-scan ultrasonography (Wallman and Adams, 1987) performed to determine refractive errors and the internal axial dimensions respectively. Anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987) under ketamine/Rhompun anaesthesia (see Appendix I).

Data analysis

Data were analyzed using nonparametric statistics. To assess the difference between treated eyes (T) and normal eyes (N) within treatment groups, the Wilcoxon matched-pairs signed-ranks test (WSRT) was used. The Mann-Whitney U-test (MWUT) was used to compare treatment effects, i.e. differences between treatment groups. For the latter analysis, treatment effects were represented as the differences between treated and normal eyes of individual animals (see Appendix I for more details).

4.1.3. Results

Ciliary nerve section

Ciliary nerve section (CNS) produced non-reactive, widely dilated pupils and accommodative paralysis; both of these effects were observed in all chicks and were maintained for the period of the study, i.e. 11 days. No accommodative response could be elicited, either with near targets or negative lenses, in CNS eyes; this contrasted with strong accommodative responses in normal eyes of the same chicks to the same stimuli. Surgery also resulted in slight, generally transient, lid inactivity. However 13 chicks were excluded from the study because of poor lid function.

CNS alone had no effect on refraction, with CNS eyes having a mean refractive error of +1.3±0.8 D compared with +1.9±0.4 D for normal eyes. However, CNS did cause significant changes to some of the measured ocular parameters (see Appendix II, Table AII.4.1, for table of treated and normal eye data). Treated eyes had significantly longer vitreous chambers compared with normal eyes, i.e. by 0.14±0.17 mm ($P < 0.01$, WSRT); for AL, the equivalent value difference was 0.13±0.21 mm ($P < 0.01$, WSRT) greater than normal. Anterior chambers of CNS groups were significantly deeper than normal; the mean ACD of treated eyes was 1.31±0.09 mm compared with 1.24±0.03 mm for normal eyes ($P < 0.05$, WSRT). An interocular difference in corneal power of −0.6±2.1 D was observed but was not statistically significant (Table 4.1.2). The surgery also resulted in significant lens thinning, with the ALT of treated eyes being 0.08±0.01 mm thinner than normal ($P < 0.01$, WSRT). While it is likely that lens thinning contributed to the ACD changes, it did not account entirely for the increase in ACD, as indicated by the poor correlation between the two measurements ($r = 0.438$, NS).

It has been suggested that changes in lens size do not necessarily lead to changes in lens power and hence refractive state (Sivak *et al.*, 1989b). However, in this case, where comparisons are made between treated and normal eyes, it is likely that the thinner lens will also be less powerful. The lens thinning is likely to be caused by equatorial stretching of the lens, the lens will be flatter and hence less powerful. Here, emmetropia was maintained by the changes in anterior and vitreous chamber depths, negating the refractive effects of a thinner lens.

Constant defocus and ciliary nerve section

Results presented in this section relate only to lens-treated eyes that also had CNS; the lenses in all cases were worn constantly. CNS, while

eliminating accommodative function, did not prevent the adaptation to artificially induced refractive errors. At day 11, after only 4 days of lens wear, there were significant differences in the refraction of chicks wearing +10 D lenses compared with – 10 D lenses ($P < 0.005$, MWUT). Constant +10 D spectacle lens wear (myopic defocus) induced an average +10.6±4.8D hyperopic shift in refraction; the refractive error of treated eyes was +12.0±4.4 D compared with +1.4±1.8 D for normal eyes ($P < 0.005$, WSRT; see Appendix II, Table AII.4.1, for treated and normal eye data and Table 4.1 2 for interocular differences). This contrasts with a large myopic shift in refraction, i.e. –6.3±2.6 D, for –10 D spectacle lens wear (hyperopic defocus); this value represents the difference between a mean refractive error of –4.8±1.5 D for treated eyes and of +1.5±1.4 D for normal eyes ($P < 0.005$, WSRT).

Constant +10 D lens wear significantly inhibited growth of the vitreous chamber; the mean VCD of treated eyes was 4.85±0.26 mm compared with 5.14±0.10 mm for normal eyes ($P < 0.005$, WSRT). As a consequence of this inhibition, axial growth of treated eyes was also less than normal, i.e. 8.03±0.27 mm compared with 8.48±0.10 mm for treated and normal eyes respectively ($P < 0.005$, WSRT). In contrast, constant –10 D lens wear, enhanced vitreous chamber growth, by 0.41±0.15 mm over the experimental period, i.e. 5.59±0.12 mm compared with 5.18±0.24 mm for treated and normal eyes respectively ($P < 0.005$, WSRT). Similarly, a large increase in mean axial growth of 0.47±0.12 mm was seen for the –10 D treated eyes compared with normal eyes ($P < 0.005$, WSRT). The effects on vitreous chamber and axial growth were significantly different for the +10 D and –10 D treatment groups (ΔVCD and ΔAL, $P < 0.005$, MWUT).

Constant +10 D lens wear resulted in anterior chamber shallowing of 0.08±0.07 mm ($P < 0.05$, WSRT), contrasting with anterior chamber deepening of 0.09±0.10 mm ($P < 0.05$, WSRT) for constant –10 D lens wear with CNS. The deepening of the anterior chamber seen with –10 D lens wear, was not significantly different from the anterior chamber deepening seen in the plano treatment group and thus is more likely to be a result of CNS rather than a specific response to the negative lenses.

The changes in ACD were reflected in the curvature of the cornea. Shallowing of the anterior chamber was coupled with corneal flattening for +10 D lens wear; the corneal power of treated eyes was 106.3±3.4 D compared with 110.9±2.4 D for normal eyes ($P < 0.01$, WSRT). Deepening of the anterior chamber was coupled with slight, but not significant, corneal steepening, i.e. +0.66±4.2 D, for –10 D lens wear.

Lens thinning occurred for both treatment groups; the lenses of treated eyes were 0.07±0.02 mm thinner than normal for constant +10 D lens wear ($P < 0.05$, WSRT), and 0.03±0.02 mm thinner than normal for constant −10 D wear. As lens thinning also occurred for the plano treatment group, it seems likely that this change was linked to the surgery and subsequent elimination of accommodation. Significantly less thinning occurred for the − 10 D treatment group compared with the control plano group ($P < 0.05$, MWUT).

Table 4.1.2. The difference in ocular parameters between treated eyes (constant lens wear with CNS) and normal eyes at day 11 (means ± SD, n = 7, 7, 9, 7).

	Treatment group			
Ocular parameter	**+10 D + CNS**	**−10 D + CNS**	**Plano + CNS**	**−10 D**
Δ Refraction (D)	+10.6±4.8***	−6.3±2.6***	−0.6±0.9	−8.6±1.6
Δ Corneal power (D)	−4.5±3.1**	+0.66±4.2	−0.6±2.1	+4.0±1.0•
Δ Anterior chamber depth (mm)	−0.08±0.07**	+0.09±0.10	+0.07±0.07	+0.06±0.14
Δ Axial lens thickness (mm)	−0.07±0.02	−0.03±0.02*	−0.08±0.01	−0.03±0.03
Δ Vitreous chamber depth (mm)	−0.29±0.17***	+0.41±0.15***	+0.14±0.17	+0.30±0.15
Δ Axial length (mm)	−0.45±0.20***	+0.47±0.12***	+0.13±0.21	+0.33±0.13•

*Differences between refractive lens groups (+10 D + CNS and −10 D + CNS) and plano treatment group significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed); differences between −10 D + CNS and −10 D lens groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test.*

Comparison of −10 D lens wear with and without CNS

In the case of the CNS group, even though lens wear was delayed slightly, adaptation was rapid, producing a greater response over the same time period than when lens wear was commenced at hatch (section 3.4). However, there was no statistically significant difference ($P < 0.05$, MWUT) between the −10 D lens groups with and without CNS for any of the ocular parameters (Table 4.1.2). However the CNS lens combination had an apparently greater effect on both VCD and AL compared with lens wear alone and this difference was significant in the case of the AL data. Negative lens wear alone also produced more corneal steepening than the combination of lens wear and CNS; this was also statistically significant ($P < 0.05$, MWUT).

Short periods of defocus and ciliary nerve section

Introducing periods of normal vision acted to decrease the size of refractive shifts for both + 10 D lens wear with CNS (myopic defocus) and –10 D lens wear with CNS (hyperopic defocus; Fig. 4.1.1, Table. 4.1.3). For + 10 D lens wear, the magnitude of hyperopic shifts in refraction systematically decreased with increased periods of normal vision. Decreasing the period of lens wear by introducing 3 hrs of normal vision per day decreased the hyperopic shift to +6.6±3.4 D; the mean refractive error of treated eyes was 7.5±2.8 D compared with +1.6±1.4 D for normal eyes ($P < 0.005$, WSRT). Refractive differences between treated and normal eyes of +4.5±2.1 D ($P < 0.01$, WSRT) and +2.7±1.1 D ($P < 0.05$, WSRT) were seen when +10 D lens wear was further reduced by 6 and 9 hrs of normal vision, respectively. Thus, short daily periods of +10 D lens wear (myopic defocus) always induced a hyperopic refractive shift, albeit small in some cases. A different picture was seen for –10 D lens wear (hyperopic defocus). Myopic shifts in refraction were produced when the –10 D lenses were worn constantly, but this effect decreased rapidly when periods of normal vision were introduced. Myopic shifts decreased from –6.3±2.6 D ($P < 0.005$, WSRT) for constant –10 D lens wear to –2.5±2.5 D ($P < 0.05$, WSRT) with 3 hrs of normal vision and to –0.4±1.7 D (not significant) and –0.2±2.7 D (not significant) with 6 and 9 hours of normal vision respectively. As CNS alone induced a low myopic shift in refraction, i.e. –0.6±2.1 D, it can be concluded that the effect of –10 D lens wear on refraction was minimal when worn for either 3 or 6 hrs per day (Fig. 4.1.1).

Refractive shifts were significantly greater than those of the control group (Plano + CNS) for all the +10 D lens treatment groups and for the constant and 3 hr normal vision –10 D treatment groups (Table 4.1.3).

The changes in ocular growth described for constant refractive defocus with CNS were always less when periods of normal vision were introduced (Fig. 4.1.1 and Fig. 4.1.2). The mean changes seen in refraction were correlated to mean vitreous chamber changes for "pooled" refractive lens treatment groups ($r = 0.838$, $P < 0.05$, +10 D + CNS; $r = 0.969$, $P < 0.02$, –10 D + CNS; Fig. 4.1.5).

Table 4.1.3. Differences between refractions of treated eyes (lens + CNS) and normal eyes for different periods of normal vision, at day 11 (mean ± SD, n = 7 all groups).

Period of normal vision	Refractive adaptation (T-N, D)	
	+10 D + CNS	−10 D + CNS
0 (hrs/day)	+10.6±4.8***	−6.3±2.6***
3	+6.6±3.4***	−2.5±2.5*
6	+4.5±2.1**	−0.4±1.7
9	+2.7±1.1**	−0.2±2.7

*Differences between refractive lens groups (+10 D and −10 D) and plano control group significant at *P < 0.05, **P < 0.01, ***P < 0.005 Mann-Whitney U-test (one-tailed).*

The inhibited growth of the vitreous chamber seen with constant +10 D lens wear was reduced in magnitude when periods of normal vision were introduced, although all the +10 D lens showed inhibited VCD growth compared with the control (Plano + CNS; Fig. 4.1.2). Thus while the control group showed exaggerated vitreous chamber growth, i.e. 0.14±0.17 mm, the +10 D lens groups recorded interocular differences of, −0.06±0.12 mm ($P < 0.01$, MWUT), −0.07±0.11 mm ($P < 0.01$, MWUT) and −0.07±0.25 mm ($P < 0.05$, MWUT) for 3, 6 and 9 hr treatment groups respectively (Fig. 4.1.2). Changes in axial growth showed strong correlation with changes in vitreous chamber growth ($r = 0.838$, $P < 0.05$) so similar trends are observed in the AL data. Axial growth was always inhibited for the +10 D groups relative to the control (Plano + CNS), even with short periods of lens wear. Growth was inhibited by 0.20±0.15 mm ($P < 0.005$, MWUT) and 0.14±0.17 mm ($P < 0.01$, MWUT) for the 3 and 6 hr treatment groups respectively, while for the 9 hr treatment group, axial growth was slightly enhanced, i.e. by 0.04±0.30 mm. The latter figure was still less than the increased growth of +0.13±0.21 mm seen for the control group although the difference was not significant (Fig. 4.1.1).

The increased growth of the vitreous chamber seen with constant −10 D lens wear decreased dramatically when periods of normal vision were introduced (Fig. 4.1.2). Treatment effects on vitreous chamber growth for short periods of −10 D lens wear were not significantly different from those of the control (Plano + CNS). The increase in vitreous chamber growth seen in the CNS group, i.e. of 0.14±0.17 mm,

compares with increases of 0.19±0.03 mm, 0.17±0.06 mm and 0.10±0.11 mm for the 3, 6, and 9 hr treatment groups respectively (Fig. 4.1.2). Changes in axial growth were again highly correlated to changes in vitreous chamber growth (r = 0.969, P < 0.02). There was no significant difference in the AL changes produced by short periods of –10 D lens wear compared with the control (Plano + CNS); increases of 0.14±0.05 mm, 0.10±0.09 mm and 0.08±0.08 mm were recorded for the 3, 6, and 9 hr normal vision treatment groups respectively, compared with 0.13±0.21 mm for the control group (Fig. 4.1.1).

Constant +10 D lens produced shallower anterior chambers than normal and this effect was still evident when 3 hrs of normal vision was introduced. However, longer periods of normal vision prevented this effect (Fig. 4.1.2). The mean anterior chamber depth of treated eyes was 1.12±0.06 mm compared with 1.21±0.05 for normal eyes for the +10 D, 3 hr treatment group (P < 0.005, WRST). For the other + 10 D lens wearing groups (6 and 9 hrs normal vision) the changes in ACD were not significantly different from those of the control group. Although the anterior chamber was deeper than normal for constant –10 D wear, this effect was not significantly different from the deepening seen with the control group (Plano + CNS), and did not change when short periods of –10 D lens wear were given. The mean changes in refraction were not significantly correlated to mean anterior chamber changes for either the +10 D or –10 D lens treatment groups (Fig. 4.1. 5).

Constant +10 D lens wear induced corneal flattening although this effect persisted for the shortest period of normal vision only, i.e. 3 hrs (Fig. 4.1.3). The mean corneal power of treated eyes was 104.4±1.6 D compared with 108.8±1.3 for normal eyes for the +10 D, 3 hr treatment group (P < 0.01, WRST). Corneal power was not significantly altered by either constant or intermittent –10 D lens wear.

Significant lens thinning was observed in the control group (Plano + CNS) and this trend was also seen in all other treatment groups. Slightly less thinning was observed with –10 D lenses worn either for 9 hrs or constantly, and +10 D lenses worn for 6 hrs (Fig. 4.1.2). These changes in ALT were not significantly correlated with mean ACD changes for either the +10 D or –10 D lens treatment groups (Fig. 4.1.4).

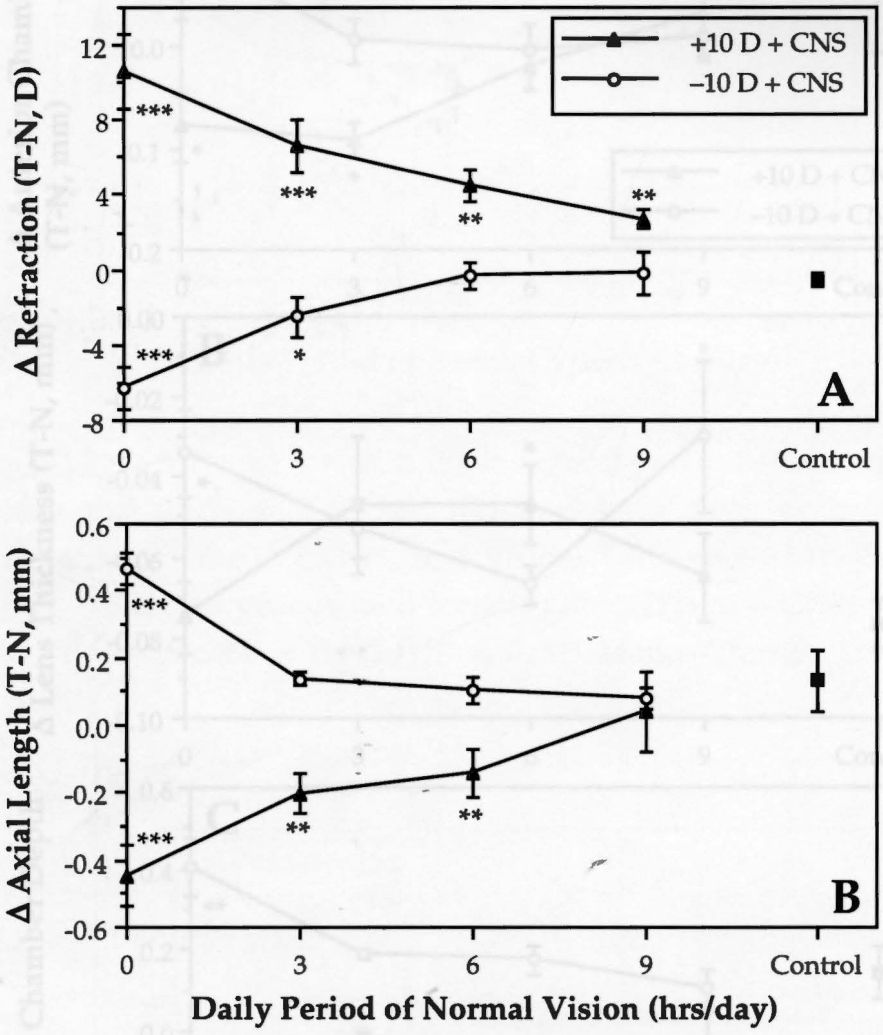


Figure 4.1.1. Differences (mean ± SE) in **A.** refraction and **B.** axial length between treated (lens and CNS) and normal eyes with daily periods of both refractive defocus and normal vision. Differences between +10 D and –10 D lens groups and control group (Plano + CNS) significant at *P < 0.05, **P < 0.01, ***P < 0.005 Mann-Whitney U-test (one-tailed).

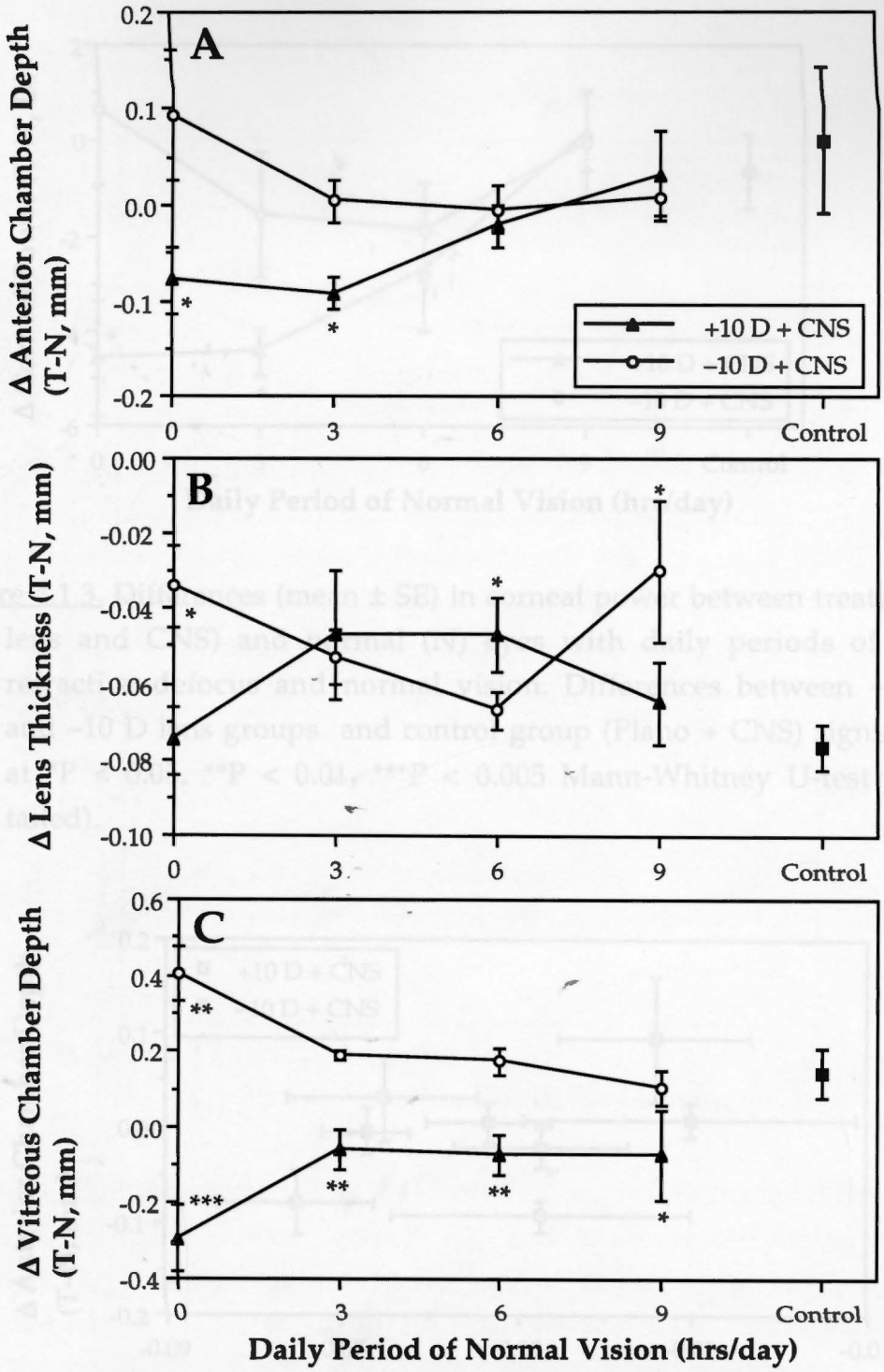


Figure 4.1.2. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T, lens and CNS) and normal (N) eyes with daily periods of both refractive defocus and normal vision. Differences between +10 D and −10 D lens groups and control group (Plano + CNS) significant at *P < 0.05, **P < 0.01, ***P < 0.005 Mann-Whitney U-test (one-tailed).

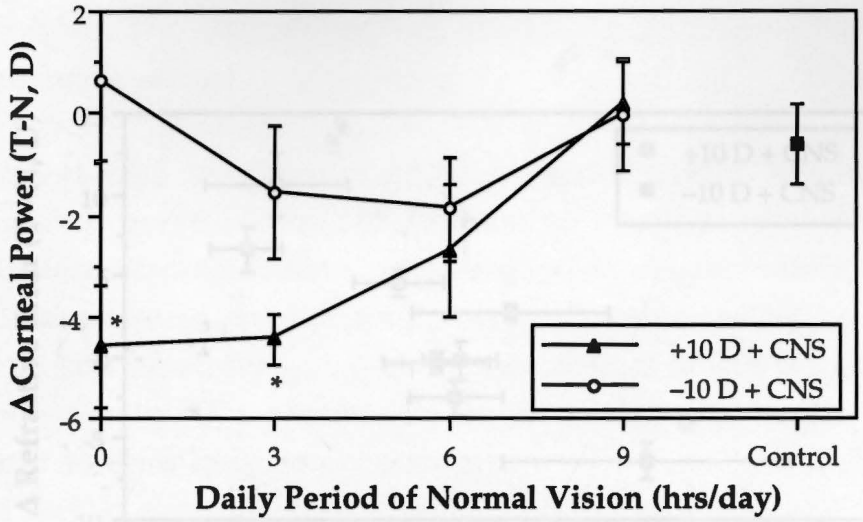


Figure 4.1.3. Differences (mean ± SE) in corneal power between treated (T, lens and CNS) and normal (N) eyes with daily periods of both refractive defocus and normal vision. Differences between +10 D and –10 D lens groups and control group (Plano + CNS) significant at *P < 0.05, **P < 0.01, ***P < 0.005 Mann-Whitney U-test (one-tailed).

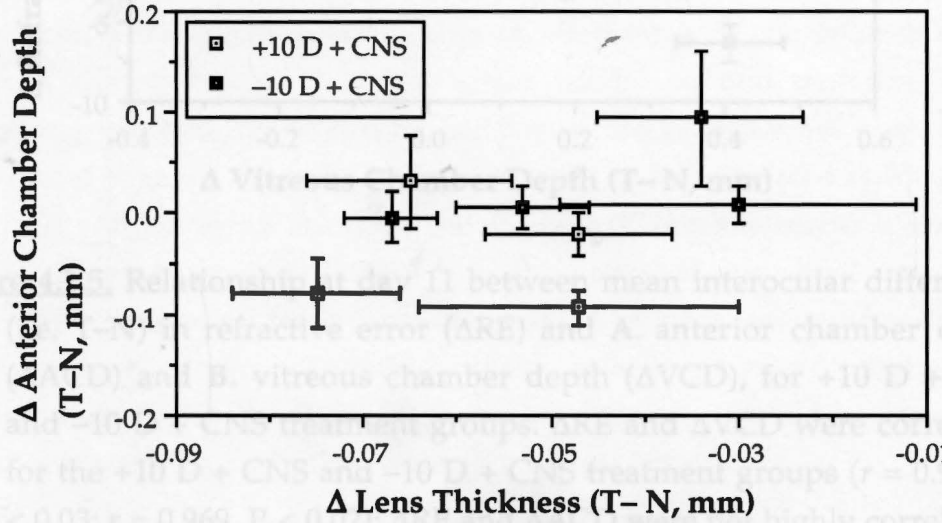


Figure 4.1.4. Relationship at day 11 between interocular, i.e. T–N, mean differences in anterior chamber depth (ΔACD) and mean differences in lens thickness (ΔLT), for +10 D + CNS and –10 D + CNS treatment groups. ΔACD and ΔLT were not significantly correlated.

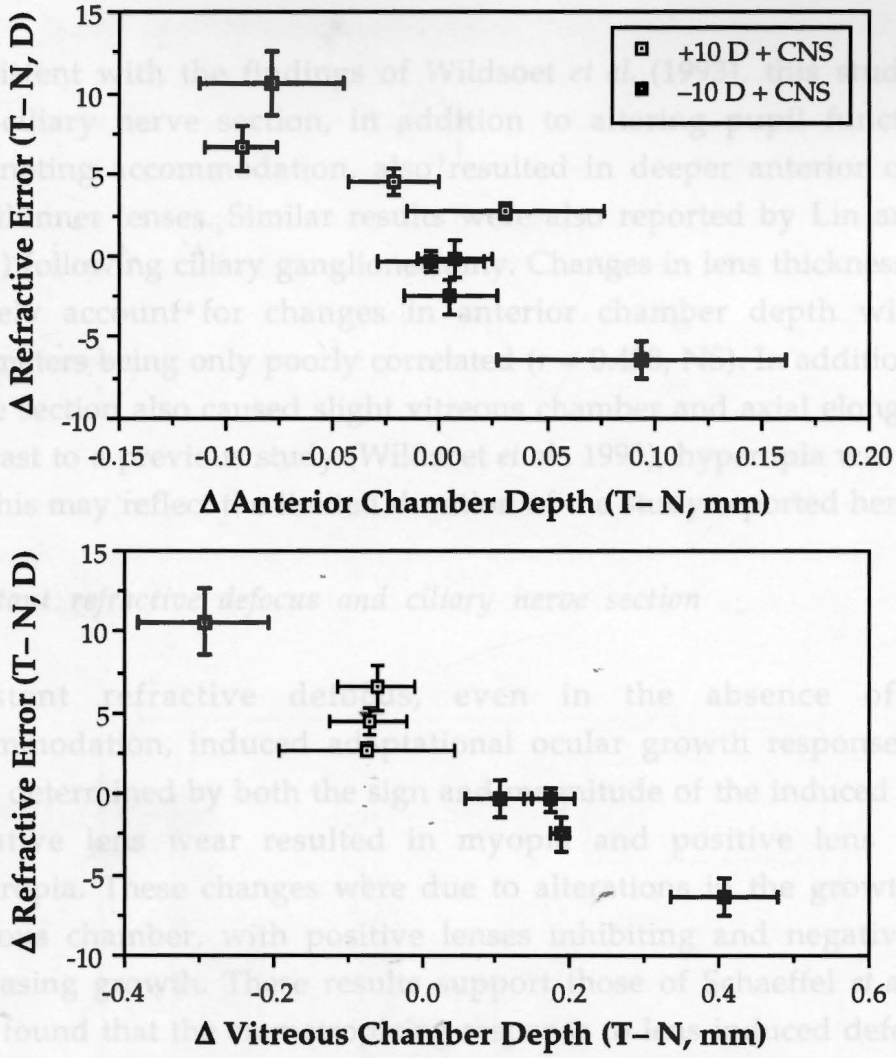


Figure 4.1.5. Relationship at day 11 between mean interocular differences (i.e. T–N) in refractive error (ΔRE) and **A.** anterior chamber depth (ΔACD) and **B.** vitreous chamber depth (ΔVCD), for +10 D + CNS and –10 D + CNS treatment groups. ΔRE and ΔVCD were correlated for the +10 D + CNS and –10 D + CNS treatment groups (r = 0.963, P < 0.03; r = 0.969, P < 0.02); ΔRE and ΔACD were not highly correlated.

4.1.4. Discussion

Ciliary nerve section

Consistent with the findings of Wildsoet *et al.* (1993), this study found that ciliary nerve section, in addition to altering pupil function and eliminating accommodation, also resulted in deeper anterior chambers and thinner lenses. Similar results were also reported by Lin and Stone (1991) following ciliary ganglionectomy. Changes in lens thickness did not entirely account for changes in anterior chamber depth with these parameters being only poorly correlated (r = 0.438, NS). In addition, ciliary nerve section also caused slight vitreous chamber and axial elongation. In contrast to a previous study (Wildsoet *et al.*, 1993), hyperopia was not seen but this may reflect the limited duration of the study reported here.

Constant refractive defocus and ciliary nerve section

Constant refractive defocus, even in the absence of active accommodation, induced adaptational ocular growth responses which were determined by both the sign and magnitude of the induced defocus. Negative lens wear resulted in myopia and positive lens wear in hyperopia. These changes were due to alterations in the growth of the vitreous chamber, with positive lenses inhibiting and negative lenses increasing growth. These results support those of Schaeffel *et al.* (1990) who found that the emmetropizing response to lens induced defocus still occurred following ablation of the Edinger-Westphal nucleus and the results of Wildsoet *et al.* (1993) who showed that emmetropization, as indicated by recovery from form-deprivation myopia, occurred following ciliary nerve section. Together, these results showing changes in refractive error in the absence of accommodation, would suggest that accommodation has a limited role in emmetropization.

Refractive defocus, periods of normal vision and ciliary nerve section

The introduction of periods of normal vision acted to decrease the magnitude of refractive shifts for both + 10 D + CNS and –10 D + CNS treatment groups. For + 10 D lens wear, hyperopic shifts in refraction systematically decreased with increased periods of normal vision. In

contrast myopic shifts in refraction were produced when the –10 D lens was worn constantly, but this effect decreased rapidly when periods of normal vision were introduced. Similarly, the changes in ocular growth described for constant refractive defocus with CNS were always less when periods of normal vision were introduced. Thus the inhibited vitreous chamber growth seen with constant +10 D lens wear was reduced in magnitude when normal vision was introduced. Reflecting the lack of refractive effect seen in all –10 D lens groups given normal vision vitreous chamber changes were also only small.

Comparison to predictions

Predictions about the expected change in refraction (see section 3.4), based on the assumption that periods of normal vision and refractive defocus have equal effect on eye growth and refraction, were made in a previously described study (section 3.4) and are also made here (Fig. 4.1.6).

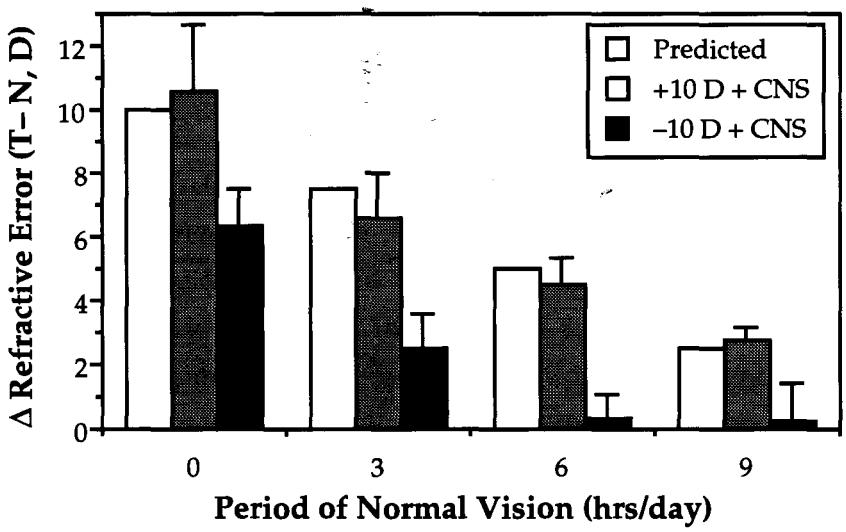


Figure 4.1.6. Predicted compared with actual changes in refraction (absolute values) for +10 D + CNS and –10 D + CNS treatment groups (means ± SE). Differences when periods of normal vision are introduced are close to predicted values for +10 D + CNS (myopic defocus) but much less than predicted for –10 D + CNS (hyperopic defocus) group.

The underlying assumption made in this analysis was that the period of lens wear and no lens wear had equivalent effects on growth, such that lenses worn for half the time would produce half the refractive change compared with that when lense were worn constantly, assuming full adaptation with constant wear. While the observed changes induced by +10 D lens wear with CNS (myopic defocus) were similar in magnitude to those predicted, the changes induced by –10 D lens wear with CNS (hyperopic defocus) were much less than predicted values the only exception involving continuous wear. This would indicate that, for +10 D lens wear, both the period of lens wear and no lens wear summate to give the resultant eye growth but that for –10 D lens wear the period of lens wear is totally negated by normal vision.

Comparison to results obtained with active accommodation

Results obtained in this section are compared with the data of section 3.4 which relate to lens wear with active accommodation. On first observation it would appear that more effective adaptation occurred in response to negative lens wear following CNS. Greater adaptation effects were observed following 4 days of lens wear with CNS compared to the equivalent period of lens wear with active accommodation and greater myopia for interrupted negative lens wear was also seen for CNS eyes. Another difference between the two experimental paradigms being compared, beside the CNS, is the earlier and longer application of lens wear in the study described in section 3.4. However, the results of the small control experiment where chicks did not undergo CNS but wore –10 D lenses from day 7 to 11, as in the study described here, also show large myopic shifts in response to continual lens wear. It would thus appear that the subtle differences in negative lens adaptation are due to negative lenses being more effective at inducing refractive changes at a slightly later age, i.e not immediately post hatching. Although greater hyperopic shifts were also seen here in response to constant +10 D lens wear; hyperopic shifts in response to the combinations of +10 D lens wear and normal vision were similar both with and without CNS for all durations of normal vision studied.

As a general finding the pattern of refractive adaptation, i.e. greater and more resistant to normal vision adaptation to +10 D lenses compared with –10 D, was still present following CNS and this would tend to rule

out accommodation being the major factor determining the pattern of adaptation observed.

Due to the elimination of accommodation, CNS eyes should have a more "constant" blur/defocus signal as accommodation may mask the imposed refractive error of the eye, at least in the case of the negative lenses. If the emmetropization process uses the defocus error as its sole cue then it would be predicted that emmetropization would be more accurate following CNS. The main trend was for CNS eyes to show changes closer to those predicted than changes for only lens treated eyes, although this effect was not great. The results would thus seem to support this model. In contrast Schaeffel *et al.* (1990) found that removing accommodation by ablation of the Edinger-Westphal nucleus resulted in greater variability of refractions in lens treated eyes. They suggested that this was due to open looping of the accommodative feedback loop and the eye having to rely solely on a retinal feedback loop for eye growth control (Schaeffel and Howland, 1988b).

While slightly greater adaptation to negative lenses was observed after eliminating accommodation, the huge difference in adaptation responses to lenses of opposite powers cannot be totally explained by accommodation effects. It had been suggested (section 3.4) that the nonlinearity of response was due to the lack of retinal blur with negative lenses because of the action of accommodation. In contrast to section 3.4, the + 10 D lenses rather than the –10 D lenses now provide greater opportunity for clear vision, at least at near distances. For –10 D lenses there is no distance at which vision is clear, unless adaptation occurs. This is accentuated by the large pupils and the decreased depth-of-focus produced by CNS. However, the nonlinearity in response to positive and negative lenses was still largely present when accommodation was abolished, arguing against the differential blur theory as outlined above as an explanation of the results.

This difference between responses is more likely to be due to some physiological difference in the growth response to defocus of opposite signs. As outlined in the previous section (3.4), an alternative interpretation in terms of hyperopic-induced growth changes being more rapid than myopic changes could account for the similarity in results observed here. This is also consistent with the data of Irving et al. (1991) showing improved compensation to negative lenses with increased duration of lens wear.

Competing eye growth signals

In the previous section the presence of both "stop" and "go" signals for controlling ocular growth were proposed, with the "stop" signal being activated by positive lens wear and the "go" by negative lens wear (Fig. 4.1.7). These signals persisted even when accommodation had been eliminated by ciliary nerve section. Thus constant +10 D lens wear, myopic defocus, here combined with CNS again produced shorter than normal eyes suggesting the activation of a "stop" signal and conversely, constant –10 D lens wear, hyperopic defocus, combined with CNS produced longer eyes, presumably by activating the "go" signal. Similarly, the pattern of signals described previously for periods of both lens wear and no lens wear, was not affected by eliminating accommodation.

The results suggests that, in the young chick eye, there are both "stop" and "go" signals for ocular growth that are activated by myopic and hyperopic defocussing errors respectively, and these can be activated in the presence or absence of accommodative input. It has been suggested (Schaeffel and Howland, 1988b) that a visual feedback system may operate to guide the eye to emmetropia, the eye responding to a signal proportional to the sign and magnitude of the ametropia. These results would suggest that accommodation is not a necessary component of this system, although undoubtedly accommodative activity must somehow be taken into account under normal circumstances.

4.1.5. Conclusion

These results suggest that, in the young chick eye, there are both "stop" and "go" signals for ocular growth that are activated by myopic and hyperopic defocussing errors respectively and that these signals can be activated in the absence or presence of accommodative input.

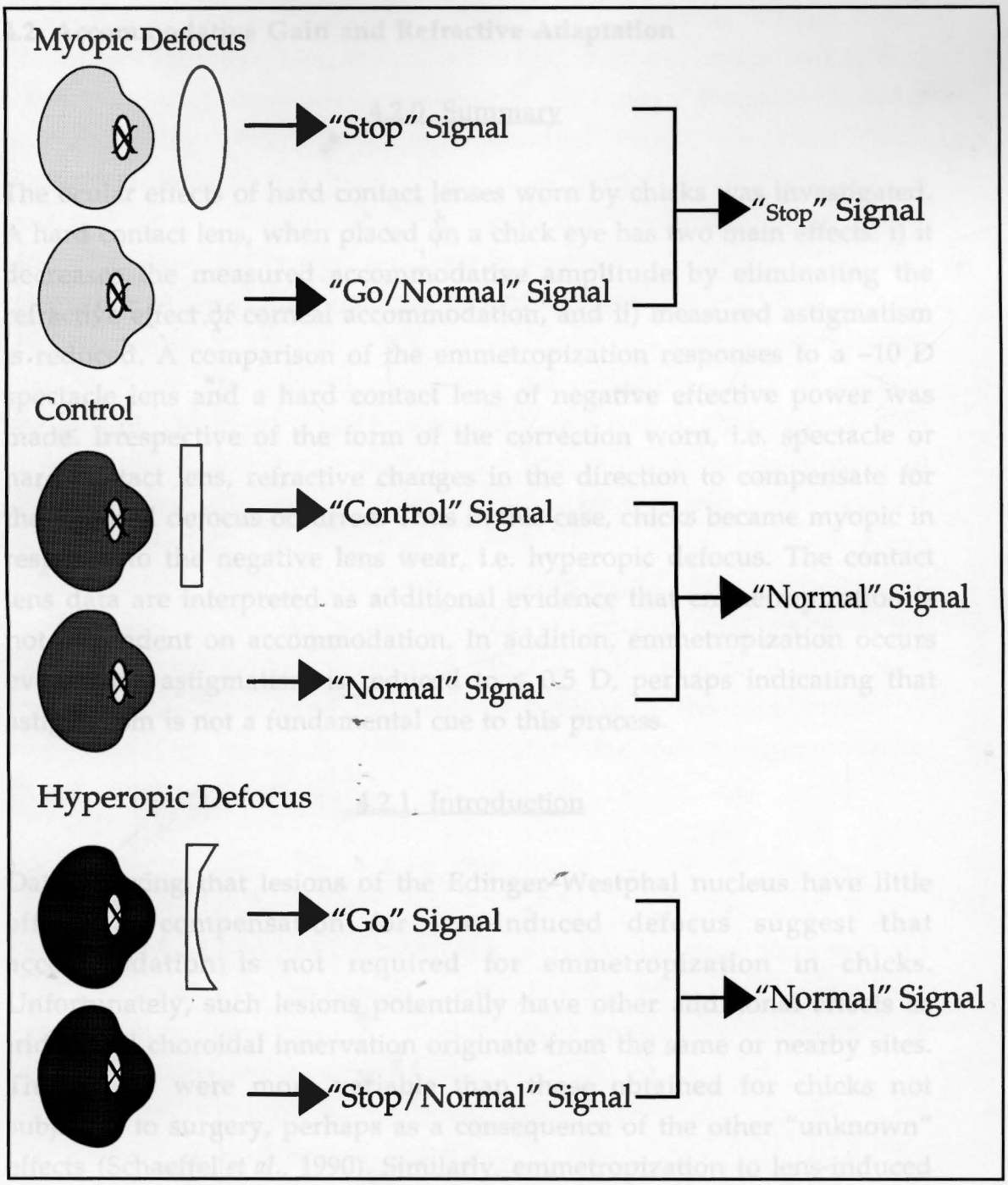


Figure 4.1.7. Competing eye growth signals in chicks following ciliary nerve section. The elimination of accommodation has been depicted as a crossed out intraocular lens. For myopic defocus (+10 D lens wear) and periods of normal vision both eye growth signals determine the resultant ocular growth, for hyperopic defocus (–10 D lens wear) the effect of normal vision dominates.

4.2. Accommodative Gain and Refractive Adaptation

4.2.0. Summary

The ocular effects of hard contact lenses worn by chicks was investigated. A hard contact lens, when placed on a chick eye has two main effects: i) it decreases the measured accommodative amplitude by eliminating the refractive effect of corneal accommodation, and ii) measured astigmatism is reduced. A comparison of the emmetropization responses to a –10 D spectacle lens and a hard contact lens of negative effective power was made. Irrespective of the form of the correction worn, i.e. spectacle or hard contact lens, refractive changes in the direction to compensate for the imposed defocus occurred. Thus in this case, chicks became myopic in response to the negative lens wear, i.e. hyperopic defocus. The contact lens data are interpreted as additional evidence that emmetropization is not dependent on accommodation. In addition, emmetropization occurs even when astigmatism is reduced to ≤ 0.5 D, perhaps indicating that astigmatism is not a fundamental cue to this process.

4.2.1. Introduction

Data showing that lesions of the Edinger–Westphal nucleus have little effect on compensation for lens-induced defocus suggest that accommodation is not required for emmetropization in chicks. Unfortunately, such lesions potentially have other additional effects as iridial and choroidal innervation originate from the same or nearby sites. The results were more variable than those obtained for chicks not subjected to surgery, perhaps as a consequence of the other "unknown" effects (Schaeffel *et al.*, 1990). Similarly, emmetropization to lens-induced defocus occurs following ciliary nerve section although end refractions tend to be hyperopic (Wildsoet *et al.*, 1993). Like Edinger–Westphal ablation surgery this surgery also has other effects such as affecting choroidal blood flow (Bill and Sperber, 1990; Fitzgerald *et al.*, 1990). Both of these techniques effectively eliminate accommodation; however the accommodation signal, i.e. no signal, is consistent with the magnitude of accommodation response, i.e. no response.

In addition to lenticular accommodation, chicks also have a corneal component to their accommodation. This was thought to be mediated by

a subdivision of the avian ciliary muscle's longitudinal bundle, called Crampton's muscle, which inserts at the corneo-scleral border and which, when contracted, increases corneal curvature, thereby increasing corneal power (Walls, 1967). In a more detailed recent study of chick accommodation, it has been shown that corneal accommodation is mediated by a contraction of the anterior ciliary muscles pulling on the inner lamella of the cornea, resulting in flattening of the peripheral and steepening of the central corneal curvature (Glasser *et al.*, 1993). A hard contact lens, when applied to the cornea, effectively neutralizes the front surface of the cornea and eliminates the refractive effect of corneal accommodation. Hard contact lenses are thus a way of changing the gain of the accommodative system. With the lens in place, the chick can still accommodate, although the net response in dioptric terms will be reduced by the extent of the corneal contribution, for any given accommodative "effort". The accommodation signal and accommodation response are no longer equivalent, the accommodation signal being higher than the refractive response. In the work described here, hard contact lenses were used as a way of decreasing the accommodative amplitude in an attempt to better understand the role of accommodation in eye growth regulation.

4.2.2. Methods

This study was performed in two parts: part I investigated the effect of a hard contact lens on the normal chick eye, and part II used hard contact lenses to investigate the effect of eliminating corneal accommodation on refractive adaptation to minus lenses.

Part I: Ocular effects of the hard contact lens.

The effect of hard contact lenses (Fig. 4.2.1; back optic radii: 3.5 mm; lens diameter: 6.0 mm; back vertex power: 0 D) on the eyes of White Leghorn-New Hampshire cross chicks and on accommodation was investigated by measuring, using retinoscopy, both the refraction and accommodative amplitude of the chick, with and without the contact lens in place. Accommodation was induced by topical application of 0.4% nicotine tartrate (2 to 3 drops) to the cornea. Nicotine induces accommodation in chicks by acting on the nicotinic receptors present in chick striated ciliary muscle (Pumphrey, 1961; Pilar *et al.*, 1987). It has a maximal effect 4-5 mins

after application and a sharp decrease in effect after 10 mins (Troilo and Wallman, 1987). The refraction of the chick was measured without and then with the contact lens, nicotine drops inserted, and the measurements repeated after a time interval of 4 mins. In addition, corneal curvature was measured, using photokeratometry, before and after insertion of nicotine drops to compare the magnitude of the changes in corneal curvature with accommodation. The measurements were performed on one eye of 6 normal chicks on days 2, 7, 10 and 14. The contact lens was worn only during measurements.

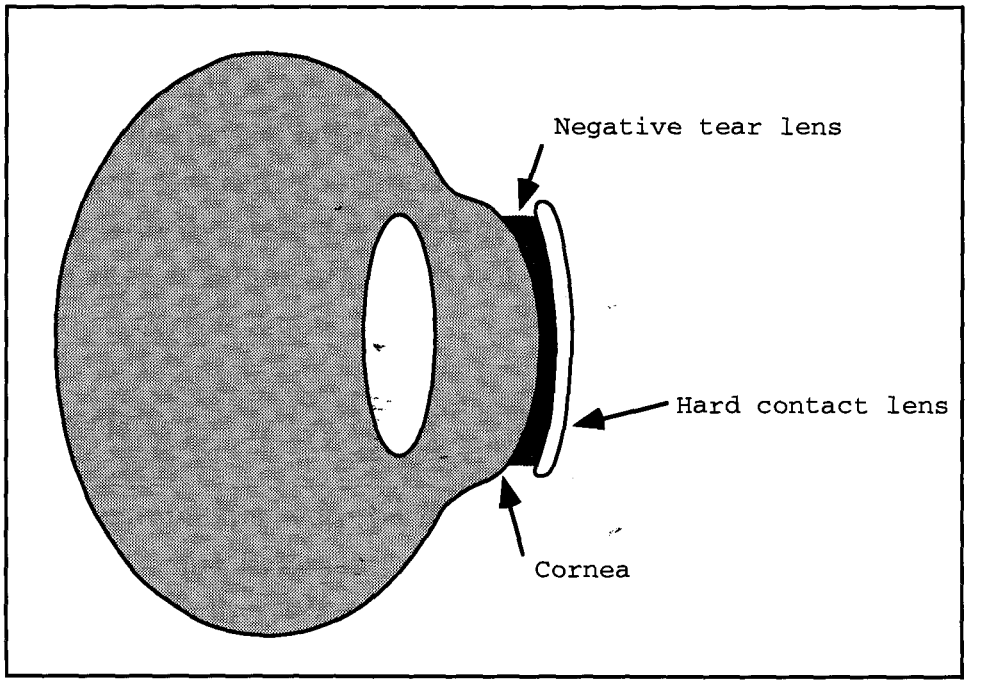


Figure 4.2.1. Schematic representation of the hard lens on a chick eye. The formation of a negative tear lens by a hard contact lens; the fluid lens acts to neutralize both corneal accommodation and astigmatism.

Part II: Contact lens compared with spectacle lens effect on ocular growth

White Leghorn-New Hampshire cross chicks were reared with either a hard contact lens (back optic radii: 3.5 mm; lens diameter: 6.0 mm; back vertex power: 0 D) or a -10 D spectacle lens. Hard contact lenses (HCL) had to be fitted large and flat for stability; the nictitating membrane of the chick passed underneath the lens. The spectacle lens (SL) was applied to the eye by means of velcro, one side being glued to the lens and the other glued to the feathers surrounding the eye of the chick. Chicks wore the hard contact lenses or -10 D spectacle lenses monocularly from day 2 to day 10.

Chicks were raised in temperature controlled enclosures, with food and water provided *ad libitum* , and were exposed to a 10 hr light/ 14 hr dark diurnal light cycle, with lights on at 8 am and off at 6 pm. The hours of light were reduced from the normal 12 to 10, for practical reasons associated with the need for constant monitoring of the HCL chicks. A light intensity of 250 lux at the level of the food trough was provided by overhead fluorescent lights.

Measurements

Ocular measurements were performed at days 5 and 10. Chicks were anaesthetized using halothane and retinoscopy (non-cycloplegic) and A-scan ultrasonography (Wallman and Adams, 1987) performed in a dim room to determine the refractive error and axial dimensions, respectively. Anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured by infrared-photokeratometry (Schaeffel and Howland, 1987) under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed-ranks test (WSRT) was used. The Mann-Whitney U-test (MWUT) was used to compare the effects of different lens types (see Appendix I for more details).

4.2.3. Results

Part I: Ocular effects of the hard contact lens

Refractive power

Normally, the chicks had low hyperopic refractions, i.e. +3.7±1.2 D at day 2 decreasing to +0.3±0.6 D by day 14 (Fig. 4.2.2). A large hyperopic shift was measured with the HCL in place, i.e. +19.9±0.7 D hyperopia at day 2 decreasing to +7.7±0.7 D at day 14. The difference in refractive state measured with and without the HCL reflected the tear lens power; as the hard lens' back vertex power was 0 D, the hyperopic shift could only be attributed to the presence of a large negative tear film between the HCL and cornea. Tears trapped between the back surface of the lens and the front surface of the cornea formed the fluid lens which was verified using fluorescein (Fig. 4.2.1). The power of the fluid lens varied from an average –16.2 D at day 2, to –7.4 D at day 14; thus the hard lens on the eye induced high hyperopic defocus. The decrease in fluid lens power with age was due to the front surface of the cornea flattening with ocular development.

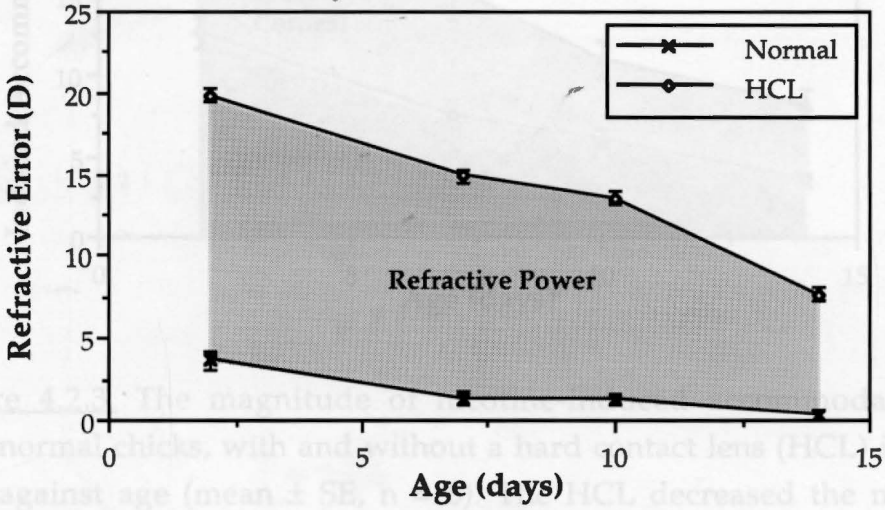


Figure 4.2.2. Refractions of normal chicks with (HCL) and without (N) a hard contact lens on the eye (mean ± SE, n = 6). The refractive power of the hard contact lens/fluid lens combination is the difference between the two successive refractions made with and without the lens in place. The HCL on the eye had high negative refracting power which decreases with age due to decreases in negative tear lens power.

Effect on accommodation

Nicotine stimulated 21.0±2.4 D of accommodation in 2 day old chicks; the nicotine-induced response decreased systematically with age to 8.3±2.2 D by day 14 (Fig. 4.2.3). With the HCL in place, the measured response to nicotine was reduced, to 12.6±2.2 D at day 2 and only 3.6±0.9 D at day 14. The lower measured accommodation in the latter instance was presumed to be attributed to corneal neutralization by the HCL, leaving only the lenticular contribution to accommodation. On this basis, it would appear that corneal accommodation made up 40%, i.e. less than half, of the total accommodation at day 2, the percentage gradually increased to 56%, at day 14, i.e. more than half the total accommodation was corneal in origin by day 14. The corneal contribution to accommodation was verified directly as described in the next section.

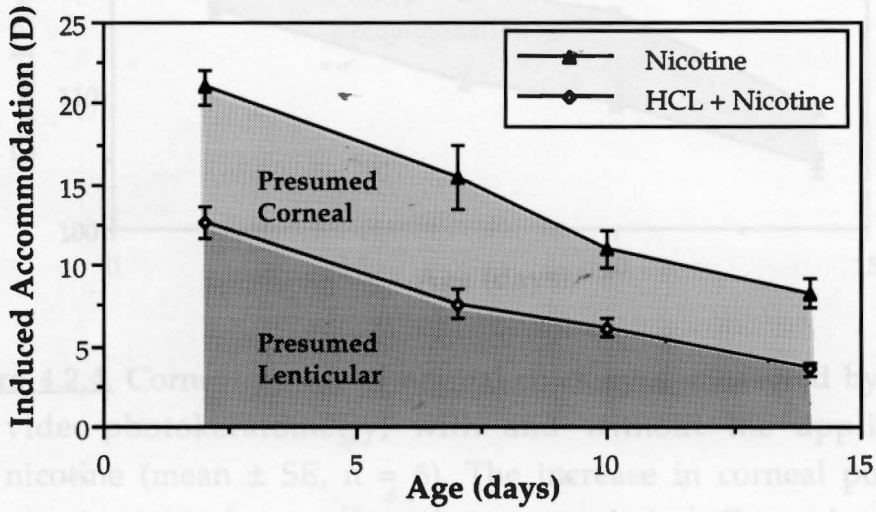


Figure 4.2.3. The magnitude of nicotine-induced accommodation, for normal chicks, with and without a hard contact lens (HCL) is plotted against age (mean ± SE, n = 6). The HCL decreased the measured accommodative amplitude, presumably by neutralizing the refractive effects of corneal accommodation.

Effect on corneal accommodation

The mean power of the cornea, as measured by photokeratometry, was 116.1±1.5 D at day 2, decreasing systematically to 104.6±2.2 D by day 14.

Following nicotine insertion, corneal power increased to 123.6±4.4 D and 108.8±1.2 D at day 2 and 14 respectively (Fig. 4.2.4). The difference between the two measurements provides a measure of the magnitude of corneal accommodation. Corneal accommodation decreased with age from 7.5 D at day 2, to 6.8 D at day 7, 6.7 D at day 10 and 4.2 D at day 14.

These values for days 2 and 14 correspond closely with differences in refractive accommodation measured with and without the HCL, thus confirming the speculation that the measured difference in accommodation with and without the HCL is due to the corneal contribution to accommodation being neutralized by the contact lens.

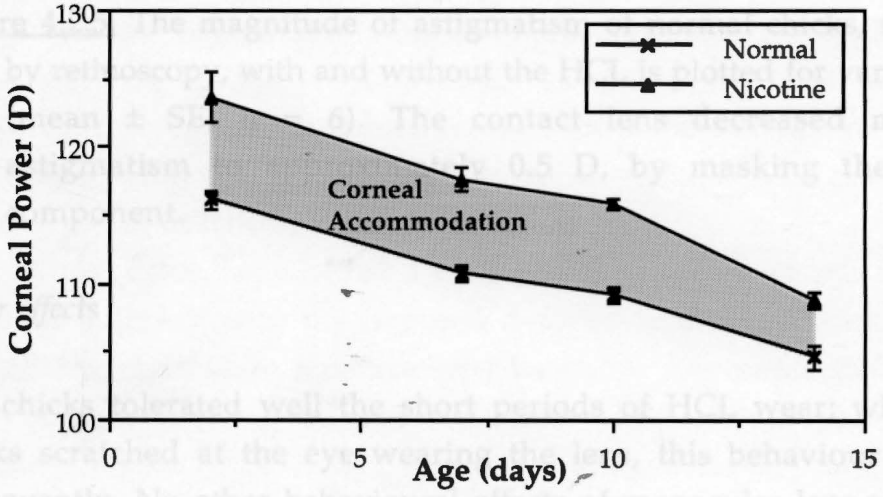


Figure 4.2.4. Corneal power of normal chick eyes, measured by infrared-video-photokeratometry, with and without the application of nicotine (mean ± SE, n = 6). The increase in corneal power with nicotine was due to corneal accommodation. Corneal power and accommodation decreased with age.

Effect on astigmatism

Not surprisingly, the HCL masked corneal astigmatism (Fig. 4.2.5). Refractions with the contact lens in place were more spherical; chicks had 2.5 D to 4 D of measured refractive astigmatism which reduced to ≤ 0.5 D when measured with a HCL in place. This result also implies that in the chick, nearly all of the astigmatism is due to the front surface of the cornea.

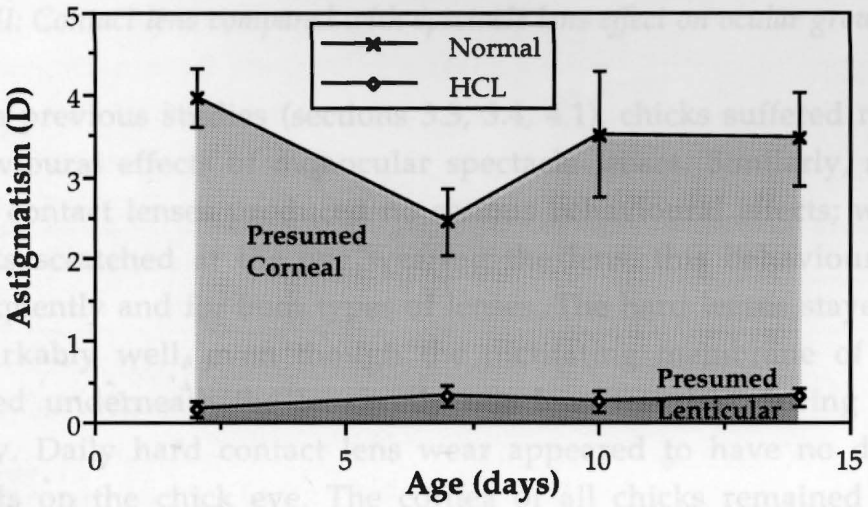


Figure 4.2.5. The magnitude of astigmatism of normal chicks, measured by retinoscopy, with and without the HCL is plotted for varying ages (mean ± SE, n = 6). The contact lens decreased measured astigmatism to approximately 0.5 D, by masking the corneal component.

Other effects

The chicks tolerated well the short periods of HCL wear; while some chicks scratched at the eye wearing the lens, this behaviour occurred infrequently. No other behavioural effects of monocular lens wear were seen.

Summary of lens effects

In summary, a hard contact lens when placed on a chick eye has two main effects: i) it will decrease measured accommodative amplitude by eliminating the refractive effect of corneal accommodation, and ii) measured astigmatism will be reduced (Table 4.2.1).

Table 4.2.1. Summary of the HCL effects.

Parameter/ Component	Lens effect
Measured accommodation	Decreased
Corneal accommodation	Neutralized
Corneal astigmatism	Neutralized

Part II: Contact lens compared with spectacle lens effect on ocular growth

As in previous studies (sections 3.3, 3.4, 4.1), chicks suffered no obvious behavioural effects of monocular spectacle lenses. Similarly, monocular hard contact lenses produced no serious behavioural effects; while some chicks scratched at the eye wearing the lens, this behaviour occurred infrequently and for both types of lenses. The hard lenses stayed in place remarkably well, even though the nictitating membrane of the chick passed underneath the lens; only one lens was lost during the entire study. Daily hard contact lens wear appeared to have no detrimental effects on the chick eye. The cornea of all chicks remained clear and corneal clouding and/or eye irritation were never seen.

In the case of spectacle-lens (SL) imposed defocus, the applied power of the lens was always –10 D. This contrasts with the hard contact lens (HCL) tear layer system which decreased in "effective" power from –13.1±3.0 D at day 5 to –8.9±2.3 D at day 10. The effective power of the HCL was derived as described in the previous section.

In both cases, i.e. whether a spectacle or hard contact lens was worn, refractive adaptation to the imposed defocus occurred, chicks becoming myopic in response to negative lens wear, i.e. hyperopic defocus (Fig. 4.2.6). Chicks wearing the spectacle lens were on average –4.0±4.2 D myopic ($P < 0.01$, WSRT) compared with normal contralateral eyes at day 5, whereas those wearing the hard contact lens were –5.8±3.6 D myopic ($P < 0.005$, WSRT). Myopia increased slightly with continued wear to –4.1±2.3 D ($P < 0.005$, WSRT) and –6.3±2.4 D ($P < 0.005$, WSRT) respectively at day 10. There was no significant difference in the magnitude of the myopic shift for the different lens types at day 5. At day 10, the myopic shift was significantly greater for HCL wear compared with SL wear ($P < 0.05$, MWUT).

There was no significant difference in the magnitude of lens adaptation for the two lens types at day 5; approximately 40% adaptation was achieved by day 5 for both groups. When the decrease in effective power of the HCL with age was taken into account at day 10, the magnitude of lens adaptation in percentage terms was significantly greater for the hard contact lens, i.e. approximately 70%, compared with 50% for the spectacle lens ($P < 0.01$, MWUT; Fig. 4.2.6). Changing the gain of the accommodative system, by eliminating corneal accommodation with a HCL, did not prevent adaptation to hyperopic defocus; adaptation was in fact greater when the defocus was produced by a hard contact lens.

Table 4.2.2. Ocular changes produced by –10 D spectacle lens (SL) and negative hard contact lens (HCL) wear. The differences between treated (T) and normal (N) eyes are shown (mean ± SD) for both days 5 and 10.

Ocular parameter	Spectacle lens		Hard contact lens	
	day 5, n=8	day 10, n=7	day 5, n=8	day 10, n=6
Δ Refraction (D)	–4.0±4.2	–4.1±2.3*	–5.8±3.6	–6.3±2.4
Δ Corneal power (D)	+0.35±2.8	–1.9±2.6*	–0.24±3.0	+0.74±3.1
Δ Anterior chamber depth (mm)	+0.02±0.03	–0.02±0.05	–0.01±0.04	+0.01±0.05
Δ Lens thickness (mm)	–0.01±0.01	–0.01±0.03	+0.01±0.02	+0.01±0.02
Δ Vitreous chamber depth (mm)	+0.19±0.08*	+0.21±0.1*	+0.33±0.13	+0.42±0.09
Δ Axial length (mm)	+0.20±0.08*	+0.18±0.1*	+0.33±0.14	+0.44±0.09

*Differences between SL and HCL groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).*

Hyperopic defocus produced by –10 D spectacle lens wear increased vitreous chamber growth by 0.19±0.08 mm (P < 0.01, WSRT) and 0.21±0.1 mm (P < 0.01, WSRT), at days 5 and 10 respectively (Fig. 4.2.7) and there was a parallel increased axial growth, of 0.20±0.08 mm (P < 0.01, WSRT) and 0.18±0.1 mm (P < 0.01, WSRT; Fig. 4.2.6), at days 5 and 10 respectively. Likewise, hyperopic defocus produced by hard contact lens wear resulted in increased vitreous chamber growth, reaching 0.33±0.13 mm (P < 0.005, WSRT) and 0.42±0.09 mm (P < 0.005, WSRT), at day 5 and 10 respectively. Axial growth increased here by 0.33±0.14 mm (P < 0.005, WSRT) and 0.44±0.09 mm (P < 0.005, WSRT) respectively. The changes in vitreous chamber and axial growth were significantly greater for the HCL compared with SL treatment group, at both measurement points (all, P < 0.05, MWUT)

Anterior chamber growth was not significantly altered by lens wear of either type and axial lens thickness was likewise unaffected (Fig. 4.2.7).

Spectacle lens wear resulted in slight corneal flattening, –1.9±2.6 D at day 10 ($P < 0.05$, WSRT); this effect was not seen here with hard contact lens wear. Corneal flattening has been reported with soft contact lens wear (Irving *et al.*, 1991). However, in contrast to the daily wear protocol used in the current study, the soft lenses were worn continually. It has recently been shown that the chick cornea is being constantly deformed by the action of the nictitating membrane and is particularly resiliant to permanent deformation (Chew *et al.*, 1994). It thus seems more likely that the reported corneal effect of soft lens wear (Irving *et al.*, 1991) was a physiological response due to oxygen deprivation rather than a result of mechanical deformation. As the hard contact lenses were removed at night, this would explain the absence of any equivalent corneal effect in the study reported here.

Predicted changes in refraction based on ocular parameter changes

Predictions of changes in refraction based on measured changes in anterior and vitreous chamber depths were similar to those measured using retinoscopy at day 5. At day 10, the measured changes were under-estimated for SL and overestimated for HCL (Table 4.2.3). Vitreous chamber elongation contributed most to the measured refractive error.

Table 4.2.3. Predicted (based on ocular parameter changes) compared with measured changes in refractive error (RE) for spectacle lens (SL) and hard contact lens (HCL) treatment groups at day 5 and 10.

	Spectacle lens		**Hard contact lens**	
	day 5	day 10	day 5	day 10
Measured Δ RE (D)	–4.0±4.2	–4.1±2.3	–5.8±3.6	–6.3±2.4
Δ RE ACD (D)	–0.58	+0.58	–0.29	–0.29
Δ RE VCD (D)	–3.00	–3.95	–6.21	–7.91
Measured Δ CP (D)	+0.35±2.8	–1.9±2.6	–0.24±3.0	+0.74±3.1
Predicted Δ RE (D)	–3.9	–1.5	–6.3	–8.9

Predicted ΔRE based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

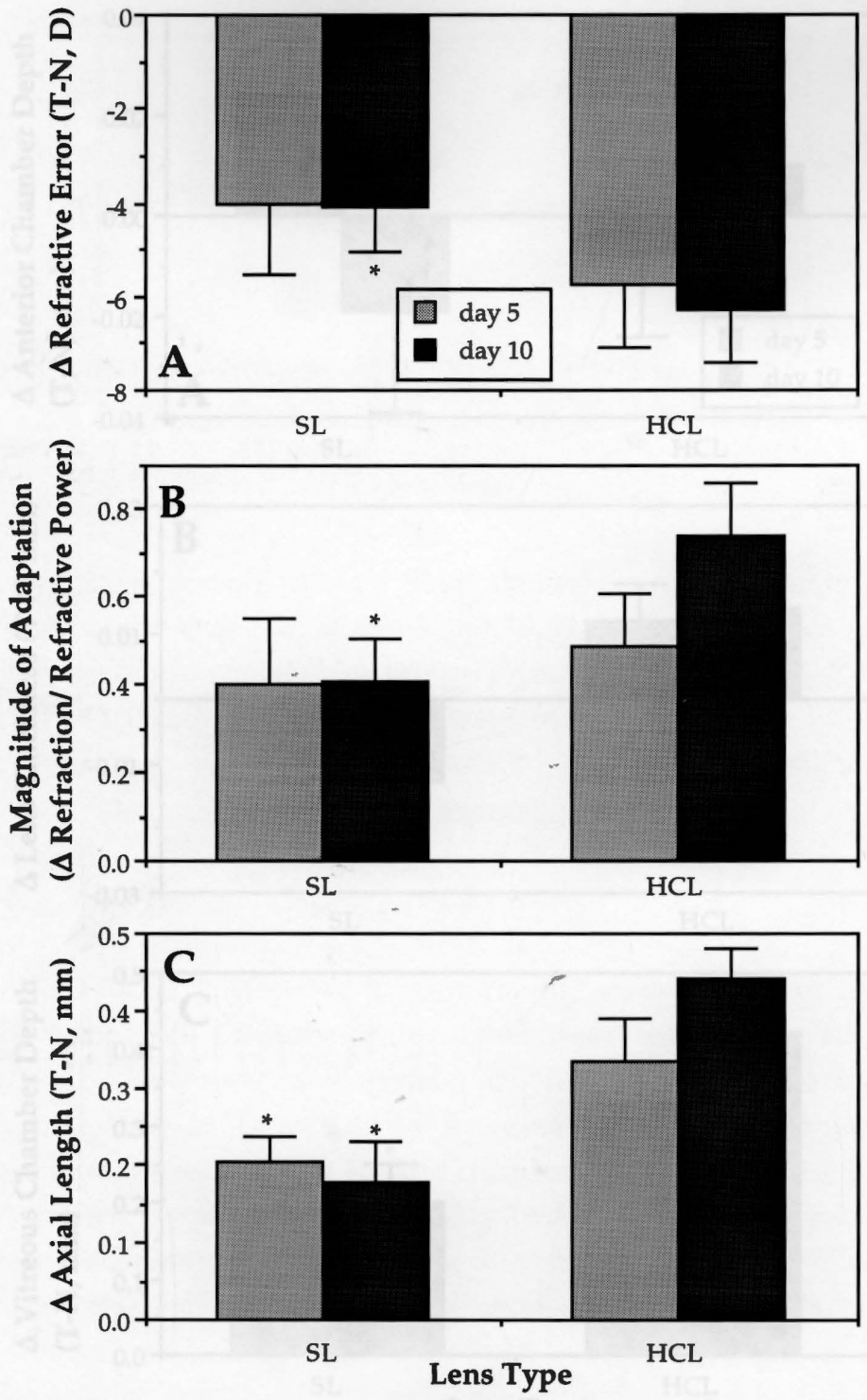


Figure 4.2.6. Differences (mean ± SE) in **A.** refraction, **B.** lens adaptation and **C.** axial length between treated (T) and normal (N) eyes, at day 5 and 10. Differences between spectacle lens and hard contact lens groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

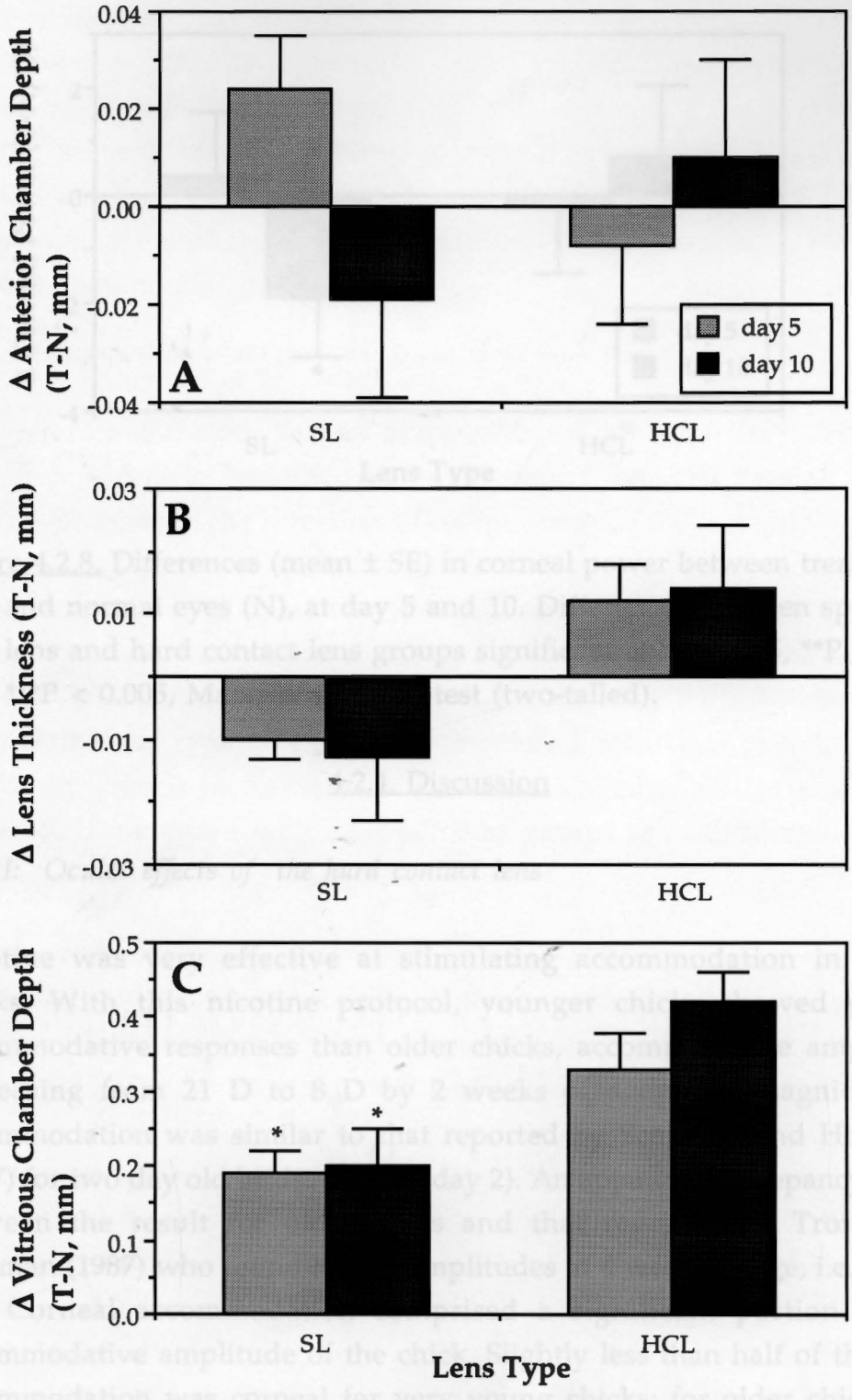


Figure 4.2.7. Differences (mean ± SE) in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes, at day 5 and 10. Differences between spectacle lens and hard contact lens groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

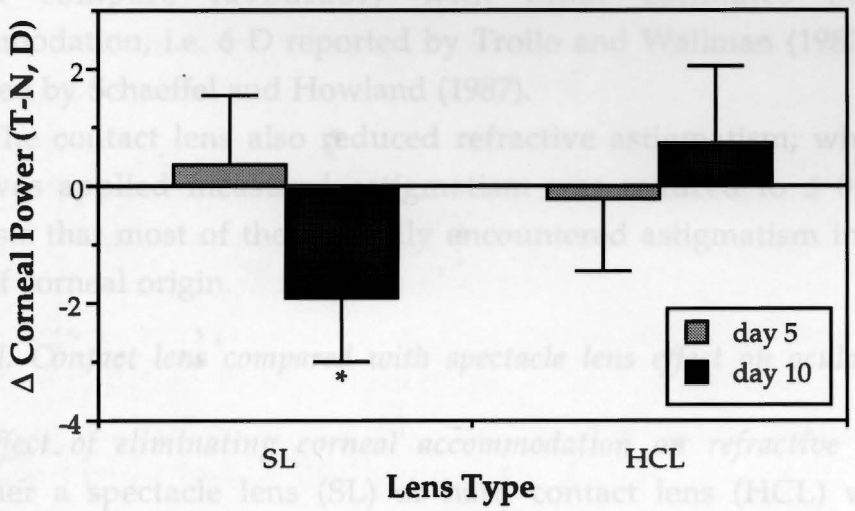


Figure 4.2.8. Differences (mean ± SE) in corneal power between treated (T) and normal eyes (N), at day 5 and 10. Differences between spectacle lens and hard contact lens groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (two-tailed).

4.2.4. Discussion

Part I: Ocular effects of the hard contact lens

Nicotine was very effective at stimulating accommodation in young chicks. With this nicotine protocol, younger chicks showed greater accommodative responses than older chicks, accommodative amplitude decreasing from 21 D to 8 D by 2 weeks of age. This magnitude of accommodation was similar to that reported by Schaeffel and Howland (1987) for two day old birds (17 D at day 2). An apparent discrepancy exists between the result for older birds and that reported by Troilo and Wallman (1987) who found higher amplitudes at 4 weeks of age, i.e. 15 D.

Corneal accommodation comprised a significant portion of the accommodative amplitude of the chick. Slightly less than half of the total accommodation was corneal for very young chicks; for older chicks the significance of corneal accommodation was increased with more than half of the total accommodation being corneal. The refractive effects of corneal accommodation were totally masked by the application of a hard lens. This reduced the measured amplitude of accommodation by approximately 7.5 D for younger chicks and by 4 D for the 2 week old chicks. The latter data represent corneal amplitudes of accommodation

which compare favourably with other estimates of corneal accommodation, i.e. 6 D reported by Troilo and Wallman (1987) and 9 D reported by Schaeffel and Howland (1987).

The contact lens also reduced refractive astigmatism; when a hard lens was applied measured astigmatism was reduced to ≤ 0.5 D. This suggests that most of the normally encountered astigmatism in the chick is of of corneal origin.

Part II: Contact lens compared with spectacle lens effect on ocular growth

The effect of eliminating corneal accommodation on refractive adaptation
Whether a spectacle lens (SL) or hard contact lens (HCL) was worn, refractive changes in the direction to compensate for the imposed defocus occurred. In response to the negative lens wear, i.e. hyperopic defocus, chicks become myopic. There was no significant difference in the effect of SL compared with HCL wear at day 5, although, by day 10, the myopic shift was significantly greater for the HCL compared with SL treatment group. Both lens types significantly increased axial eye growth, with significantly greater increases in both vitreous chamber and axial growth for the HCL compared with SL treatment groups at both measurement points.

Comparison to predictions
Eliminating the refractive effect of corneal accommodation, by the application of a hard contact lens, required the chick to exercise greater than normal lenticular accommodative effort to focus near objects. It may be argued that the lenticular accommodation system would be overwhelmed by the demand imposed by the HCL (i.e. 10 D); however, accommodative demand would have gradually reduced as ocular adaptation to the lens occurred and thus it is reasonable to assume that the accommodation system was ultimately able to compensate for the imposed defocus. If, as suggested by Schaeffel and Howland (1988b), the accommodative activity inputs into a feedback loop used in emmetropization then HCL wear should result in a greater signal than normal (Fig. 4.2.9). From this model one would predict that the greater accommodative effort and thus "improved" accommodative signal generated by the HCL should result in more effective adaptation, i.e. more myopia and perhaps even over compensation. Alternatively, if accommodation is not sustained with HCL wear, HCL wear may provide

a greater defocus signal. If retinal blur rather than accommodation is important for emmetropization, adaptation to HCL wear may be similar to or slightly improved compared with SL wear due to a more "constant" blur signal.

However, emmetropization for similar amounts of hyperopic defocus, i.e. growth in a myopic direction occurred regardless of whether the defocus was applied in spectacle or hard contact lens form. Thus again supporting a previous contention that emmetropization isn't driven by an accommodation signal. Overcompensation to constant hard contact lens wear did not occur even though this was in line with one of the models proposed and was physiologically possible; over 20 D of myopia are produced by constant form deprivation by this age (section 3.2). It could also be argued that the decrease in effective hard lens power with age would increase the likelihood of overcompensation.

In support of the retinal blur model was the result that emmetropization was faster, i.e. greater adaptation occurred, when a hard contact lens compared with a spectacle lens was used, although it could also be argued that this was a consequence of the decrease in HCL power with time in comparison to the stable power of the SL.

Accommodation feedback loop in emmetropization

Schaeffel and Howland (1988b) suggested the presence of an accommodative feedback loop involved in the regulation of eye growth (Fig. 4.2.9). Under normal circumstances, both an accommodative and a retinal feedback loop are involved. Edinger-Westphal nucleus ablation, ciliary nerve section and optic nerve section all eliminate accommodation and act to open the accommodative feedback loop. Conversely, the hard contact lenses presumably act to increase the accommodative signal. While Schaeffel and Howland (1988b) suggest that the retinal feedback loop is not important if the eye can accommodate normally, the findings here would suggest that much greater importance is placed on the retinal feedback loop than the accommodative loop. Results reported here indicate that artificially altering the accommodative signal with no concomitant alteration in refractive state does not inactivate emmetropization. If anything emmetropization is more accurate, while alteration of the retinal feedback system by the application of translucent occluders leads to a total disruption of emmetropization.

While these studies question the importance of accommodation in eye growth control it is inconceivable that accommodative activity, is not

at least taken into account as normally accommodation acts to refocus the retinal image and by its nature alters the information available to the emmetropization system. As accommodation is much faster than emmetropization the defocus error available to the emmetropization system will depend on the accuracy of accommodation.

The effect of reducing refractive astigmatism on refractive adaptation
The HCL acted to reduce drastically uncorrected astigmatism. It has been suggested that astigmatism could be used as a possible cue for controlling eye growth. The refractive separation of the line foci associated with astigmatism could for example, provide information as to direction of defocus, i.e. one of the line foci may appear clearer than the other. On the other hand, uncorrected astigmatism in childhood has been proposed as a cause of myopia (reviewed by Lyle, 1991). Van Alphen (1961) also suggested that myopia could occur from the excessive and fluctuating accommodation as the eye attempts to bring each of the line foci associated with uncorrected astigmatism in turn into focus.

These two opposing view points lead to two different models for predicting the effects of astigmatism in emmetropization. On the assumption, that astigmatism is used as a cue for defocus during emmetropization adaptation was predicted to be less accurate when astigmatism was reduced by HCL. However, compensation to applied defocus was in fact greater with the hard lens compared with the spectacle lens. It would thus seem that either extremely small amounts of astigmatism provide adequate defocus information, or alternatively that astigmatism is not used as a cue to defocus. Given that the eye's depth-of-focus is likely to mask any defocus cues provided by low levels of astigmatism the latter is more likely. As accommodation and astigmatism *per se* seem not to provide the defocus cue for emmetropization other potential cues are investigated in remaining Chapters.

4.2.5. Conclusions

In conclusion, changing the accommodative signal, by preventing corneal accommodation, does not affect emmetropization in chicks. It seems unlikely that the accommodative signal is fundamental to emmetropization. In addition, emmetropization occurs even when astigmatism is reduced to ≤ 0.5 D by the HCL; this result further implies that astigmatism is not an important defocus cue in this context.

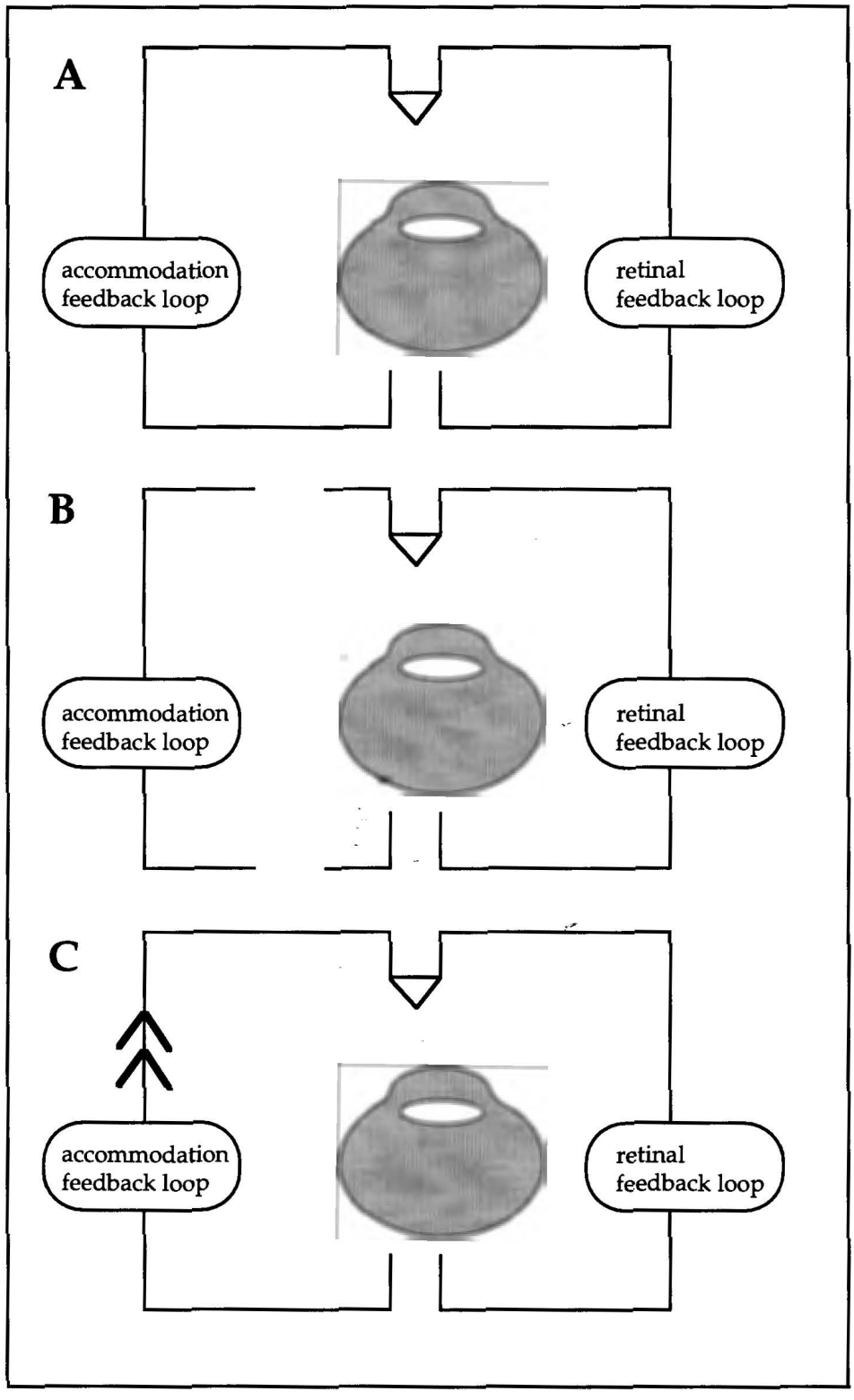


Figure 4.2.9. Feedback model for the regulation of eye growth. In **A**. the normal situation is described by accommodative and retinal feedback loops, in **B**. the accommodation feedback is open looped, i.e. rendered inoperant by either Edinger-Westphal nucleus ablation, ciliary nerve section or optic nerve section, and in **C**. the effect of hard contact lenses on the system is described, i.e. the accommodative signal is increased.

CHAPTER 5

CHROMATIC ABERRATION AND THE REGULATION OF EYE GROWTH

5.0. The Role Of Chromatic Aberration In Emmetropization

There is strong evidence that chicks use a visually guided feedback system to guide ocular growth towards an emmetropic endpoint (Schaeffel and Howland, 1988b; Wildsoet and Pettigrew, 1988; Troilo and Wallman, 1991; Schaeffel and Howland, 1991). The correlation between the ocular components required for emmetropia is extremely precise. The emmetropization mechanism must make allowances for aberrations, such as chromatic aberration, present in the eye. Due to the chromatic dispersion of the ocular media, shorter wavelengths at the blue end of the visible spectrum are more strongly refracted than longer ones, at the red end. Thus, an optimal refractive state, can only be achieved for a limited spectral range, wavelengths outside this range being slightly "out of focus".

The chick eye is sensitive to wavelengths of light between 360 nm and 700 nm (Wortel *et al.*, 1987; Schaeffel *et al.*, 1991). This makes the chick a useful model for the study of the chromatic effects on eye growth. To investigate more fully the influence of chromatic aberration in emmetropization the following studies were performed. In section 5.1, the longitudinal chromatic aberration (LCA) of the chick eye was measured using chromoretinoscopy and results compared with previous indirect estimates of LCA in chick. In section 5.2, chicks were raised in monochromatic red or monochromatic blue light to determine the wavelength sensitivity of the emmetropization process, i.e. does eye growth alter to compensate for the refractive difference between wavelengths? Finally, in section 5.3, the ability of monochromatic light to guide emmetropization in an intermittent occlusion paradigm was studied, i.e. are chromatic cues required for emmetropization?

5.1. Longitudinal Chromatic Aberration Of The Chick Eye

5.1.0. Summary

Chromoretinoscopy was used to determine the LCA of the chick eye. Using this technique, the mean LCA was 3.65±0.39 D for chicks ranging from 9 to 43 days of age. There was little variability between measurements of chicks of the same age. The measured LCA decreased slightly with development from 3.8 D at day 9 to 3.1 D at day 43. This value is higher than that previously reported for the chick.

5.1.1. Introduction

Using schematic eye data for the chick and measurements of the dispersive power of the ocular media and lens, Mandelman and Sivak (1983) determined the total power of the eye for different wavelengths of light. Using this technique, the LCA of the chick eye, between 470 nm and 680 nm, was estimated as 1.25 D (Mandelman and Sivak, 1983). This value is exceptionally low when compared with that for the human eye (2.75 D), reported in the same study across similar wavelengths. Chromoretinoscopy was used in the study described here to directly measure the LCA in chick.

5.1.2. Methods

Cycloplegic chromoretinoscopy (Bobier and Sivak, 1980) was performed, under ketamine/Rhompun anaesthesia, on the normal eye of 15 male White Leghorn-New Hampshire cross chicks varying in age from 9 to 43 days (see Appendix I). As the state of accommodation has been shown to affect LCA (Millodot and Sivak, 1973; Charman and Tucker, 1978a) cycloplegia was required. Two chicks were measured at each age, except for 22 days of age where 5 chicks were measured so that the variability between chicks could be assessed. Refractions were measured, as in conventional retinoscopy, for 7 different wavelengths of light, between 420 nm and 656 nm, and also for white light. The chromatic defocus for each wavelength was calculated by subtracting the refraction obtained for white light from that obtained using the stated wavelength. The

magnitude of LCA was determined as the difference in measurements between 420 nm and 656 nm.

5.1.3. Results

Chicks were relatively myopic for shorter wavelengths and relatively hyperopic for longer wavelengths. Using this chromoretinoscopy technique the mean LCA, between 656 nm and 420 nm, for all chicks was 3.65±0.39 D. There were only slight differences in the magnitudes of LCA measured in different chicks at the same age, 3.53±0.19 D (n = 5; Fig. 5.1.2). There was also a trend for the measured chromatic aberration of the chick eye to decrease slightly with development; LCA decreased from 3.81±0.08 D at week 1 to 3.13±0.01 D at week 6 (Fig. 5.1.3) and LCA was inversely correlated with age ($r = 0.742$, $P < 0.1$).

Calculations of the magnitude of LCA based on the axial length of the chick eye at different ages were also made (Table 5.1.2). For these calculations it was assumed that the eye was filled with water and that the axial length of the eye approximated the posterior nodal distance; both of these assumptions mean that LCA may be under-estimated. Calculated LCA decreased with age from 3.7 D at week 1, for an eye size of 8.4 mm, to 2.6 D at week 6 for a larger eye of 11.8 mm. As AL increased with age, LCA decreased.

Table 5.1.1. Longitudinal chromatic aberration for chicks of different ages (mean ± SD, n = 2 except for day 22 where n = 5).

Age (days from hatching)	LCA (D)
9	3.81±0.08
15	4.18±0.26
22	3.53±0.19
29	3.93±0.61
36	3.48±0.20
43	3.13±0.01

Table 5.1.2. Calculated longitudinal chromatic aberration (between 420 nm and 656 nm) for chicks of different ages.

Age (days)	Axial length (mm)	Power std (D)	LCA (D)
8	8.4	159	3.7
15	9.3	144	3.3
22	10.0	133	3.1
29	10.7	125	2.9
36	11.3	118	2.7
43	11.8	113	2.6

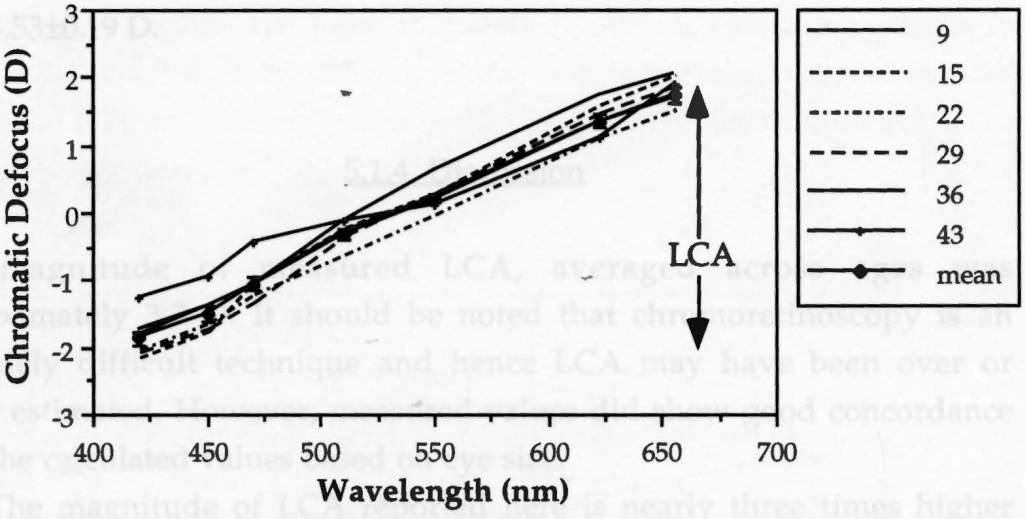


Figure 5.1.1. Longitudinal chromatic aberration (LCA) of the chick eye measured by cycloplegic chromoretinoscopy. The chick eye possesses 3.65 D of chromatic aberration between 420 nm and 656 nm, averaged across all ages. The mean chromatic defocus data comes from 9, 15, 22, 29, 36 and 43 day old chicks; n = 2 chicks for all ages except day 22 where n= 5. The mean for all chicks (± SE) at each wavelength is also plotted.

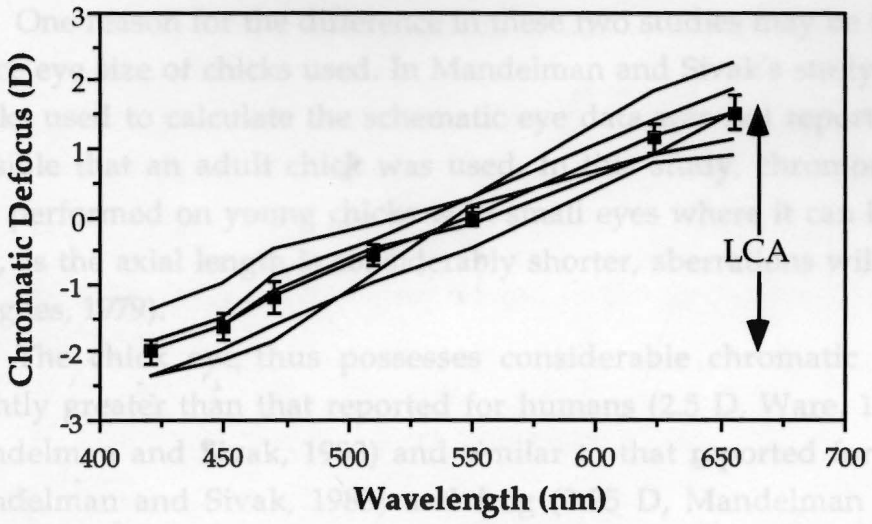


Figure 5.1.2. Longitudinal chromatic aberration (LCA) of the chick eye, measured on one eye of 5 different chicks at 22 days of age; individual defocus data for each wavelength and mean values (± SE) are shown. The average chromatic aberration for the 5 chicks was 3.53±0.19 D.

5.1.4. Discussion

The magnitude of measured LCA, averaged across ages was approximately 3.7 D. It should be noted that chromoretinoscopy is an extremely difficult technique and hence LCA may have been over or under estimated. However, measured values did show good concordance with the calculated values based on eye size.

The magnitude of LCA reported here is nearly three times higher than that reported by Mandelman and Sivak (1983). Using schematic eye data for the chick and measurements of the dispersive power of the ocular media and lens, they determined the total power of the eye for different wavelengths of light and, indirectly, the LCA for the chick eye; between 470 nm and 680 nm, LCA was estimated to be an extremely low 1.25 D. Figure 5.1.3 compares the data obtained in this study to those of Mandelman and Sivak (1983). Extrapolating the results in the current study in the long wavelength direction the comparable value over the same wavelength range was 2.8 D. The biggest differences from findings in the current study occur for long wavelengths, with less chromatic defocus being reported by Mandelman and Sivak (1983).

One reason for the difference in these two studies may be the age and hence eye size of chicks used. In Mandelman and Sivak's study, the age of chicks used to calculate the schematic eye data was not reported but it is possible that an adult chick was used. In this study, chromoretinoscopy was performed on young chicks with small eyes where it can be expected that, as the axial length is considerably shorter, aberrations will be greater (Hughes, 1979).

The chick eye thus possesses considerable chromatic aberration, slightly greater than that reported for humans (2.5 D, Ware, 1982; 2.75 D Mandelman and Sivak, 1983) and similar to that reported for rat (4.2 D, Mandelman and Sivak, 1983) and frog (3.25 D, Mandelman and Sivak, 1983). This means that, under normal viewing situations, there will be a refractive difference of 3.7 D between light from the extremes of the visible spectrum. The visual significance of this result is uncertain. However, if chicks were to focus on the middle of the spectrum, blue and red light would be defocussed by as much as 1.8 D. Alternatively, as blue light focusses before red light, relatively hyperopic chicks may focus for blue light and relatively myopic chicks for red. Either way, this result merited further investigation with respect to its implication for emmetropization.

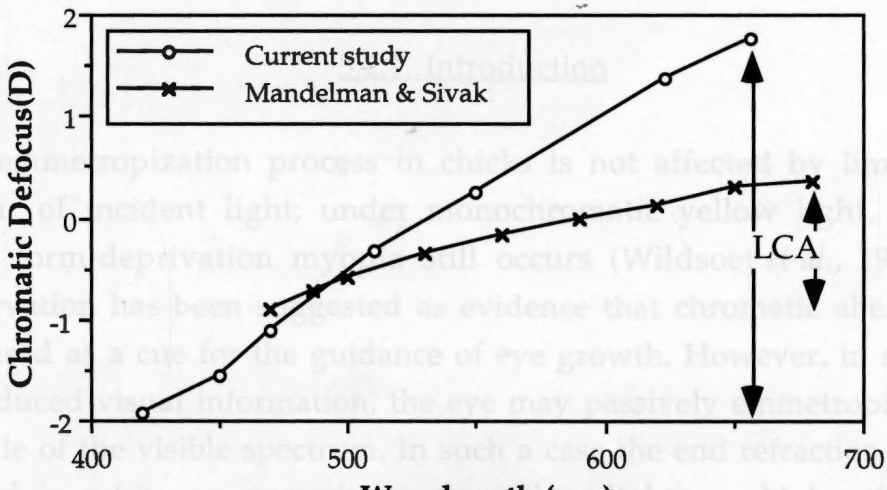


Figure 5.1.3. Comparison of chromatic aberration of the chick eye found in the current study with that estimated by Mandelman and Sivak (1983).

5.1.5. Conclusion

In conclusion, 3.65 D of LCA was measured using chromoretinoscopy for chicks ranging in age from 9 to 43 days.

5.2. The Sensitivity of Emmetropization to the Refractive Difference Of Coloured Light

5.2.0. Summary

Chicks were raised in monochromatic red or monochromatic blue light to determine the sensitivity of the emmetropization process to specific wavelengths of light, i.e. does compensation for the refractive difference between wavelengths occur? Chicks were monocularly deprived for 1 week and eye growth monitored at weekly intervals for 6 weeks. Form-deprivation myopia and recovery from myopia occurred under all lighting conditions. The end refractions suggested that the chick eye was not sensitive to the refractive difference of different wavelengths of light. Furthermore, as emmetropization still occurred under monochromatic conditions, this suggests the presence of a non-chromatic cue or cues to defocus.

5.2.1. Introduction

The emmetropization process in chicks is not affected by limiting the colour of incident light; under monochromatic yellow light, recovery from form-deprivation myopia still occurs (Wildsoet *et al.*, 1993). This observation has been suggested as evidence that chromatic aberration is not used as a cue for the guidance of eye growth. However, in situations of reduced visual information, the eye may passively emmetropize to the middle of the visible spectrum. In such a case the end refraction of chicks reared in white or monochromatic yellow light would be similar, as yellow is in the middle of the visible spectrum. Thus rearing in monochromatic yellow light does not rule out chromatic aberration as a fine tuner of ocular growth.

The chick eye possesses 3.7 D of longitudinal chromatic aberration (section 5.1). If the chick eye is sensitive to the refractive nature of coloured light and emmetropization is not dependent on LCA, a 3.7 D

difference in the refraction of chicks reared under blue light compared with those reared under red light should result, with those reared under the red light being relatively more myopic then those reared under blue light (Fig. 5.2.1). The difference in refraction is predicted on the basis that, at least with lens-induced defocus, hyperopic defocus results in increased ocular growth and myopic defocus in decreased growth (section 4.4). To investigate further the role of chromatic aberration on eye growth, chicks were reared in either monochromatic red light of wavelength 656 nm or monochromatic blue light of wavelength 420 nm; as a control chicks were also reared in white light of equivalent brightness.

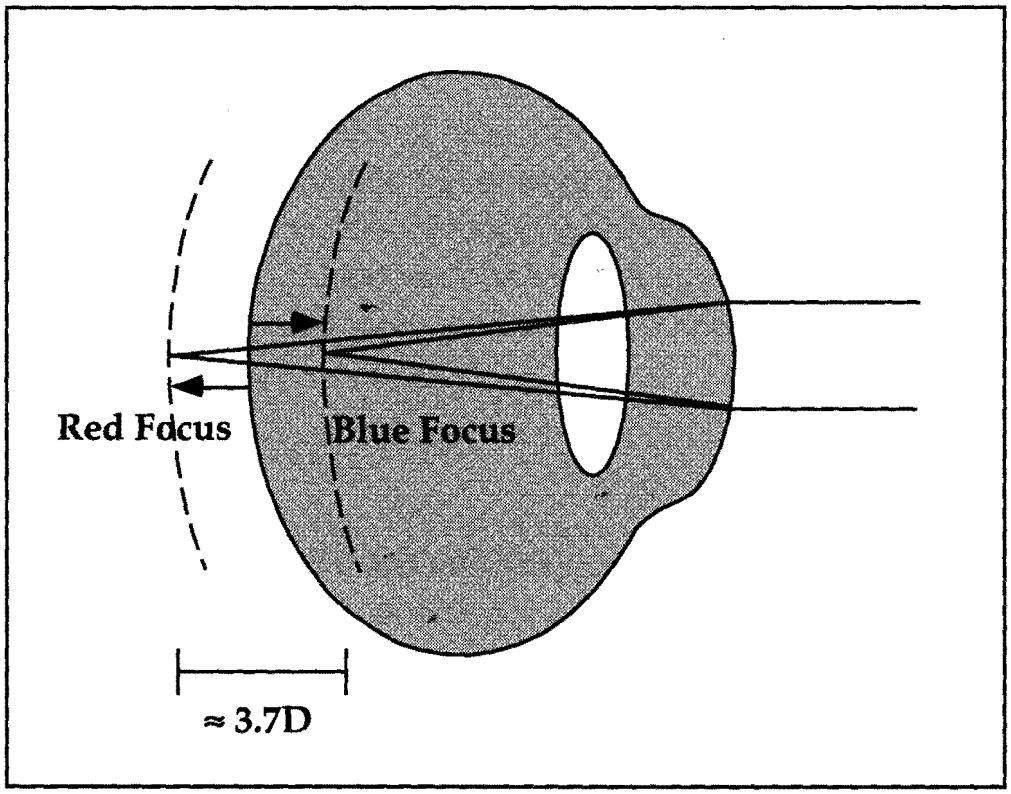


Figure 5.2.1. Model predicting how eye growth may be altered to compensate for wavelength-dependent differences in focus; chicks reared under red light should show increased eye growth and a myopic shift compared with those reared under blue light where decreased growth and a hyperopic shift should occur.

5.2.2. Methods

Treatment groups

Male, White Leghorn-New Hampshire cross chicks were reared under either monochromatic blue (420±10 nm, n = 7), monochromatic red (656±10 nm, n = 8), or white light (broad spectrum, daylight fluorescent light, n = 7). The right eyes of all chicks were occluded from day 1 for 1 week. Wavelengths at the extremes of the spectrum were chosen to obtain the greatest difference in focus. Although chicks are sensitive down to 370 nm (Wortel *et al.*, 1987; Schaeffel *et al.*, 1991), ultraviolet (UV) light does not appear to provide enough information for the emmetropization process (Rohrer *et al.*, 1992) and the role of UV sensitive receptors in normal vision is unclear. Thus 420 nm, which is within the sensitivity range of regular cone receptors, was chosen as the short wavelength condition. The monochromatic lights were produced by passing white light from Kodak projectors through interference filters (Edmunds Scientific). All stray white light was blocked. The spectral characteristics of the interference filters used were verified using a spectrophotometer (Fig. 5.2.2).

Light levels

Based on the relative luminous efficiency curve reported for the chick (Schaeffel *et al.*, 1991) the chick sees red light of wavelength 656 nm and blue light of wavelength 420 nm as nearly equally bright (Fig 5.2.3). Thus, the light intensities of the red and blue lights were matched as closely as possible. The red light source provided approximately 1 lux at the floor of the cage increasing to 10 lux 15 cm above the floor. In the case of the blue light, equivalent luminance levels ranged from 1 lux to 8 lux and, for the white light, the range of luminance was 0.5 lux to 5 lux. The light level for the blue light condition was the maximum achievable and this set the range of luminances that could be used for the other light conditions. All chicks were reared under an 8 hr light/ 16 hr dark light cycle and were provided food and water *ad libitum*. The diurnal light cycle was altered from the usual 12 hr/12 hr cycle to decrease "wear and tear" on the projectors and to limit the number of light bulbs used.

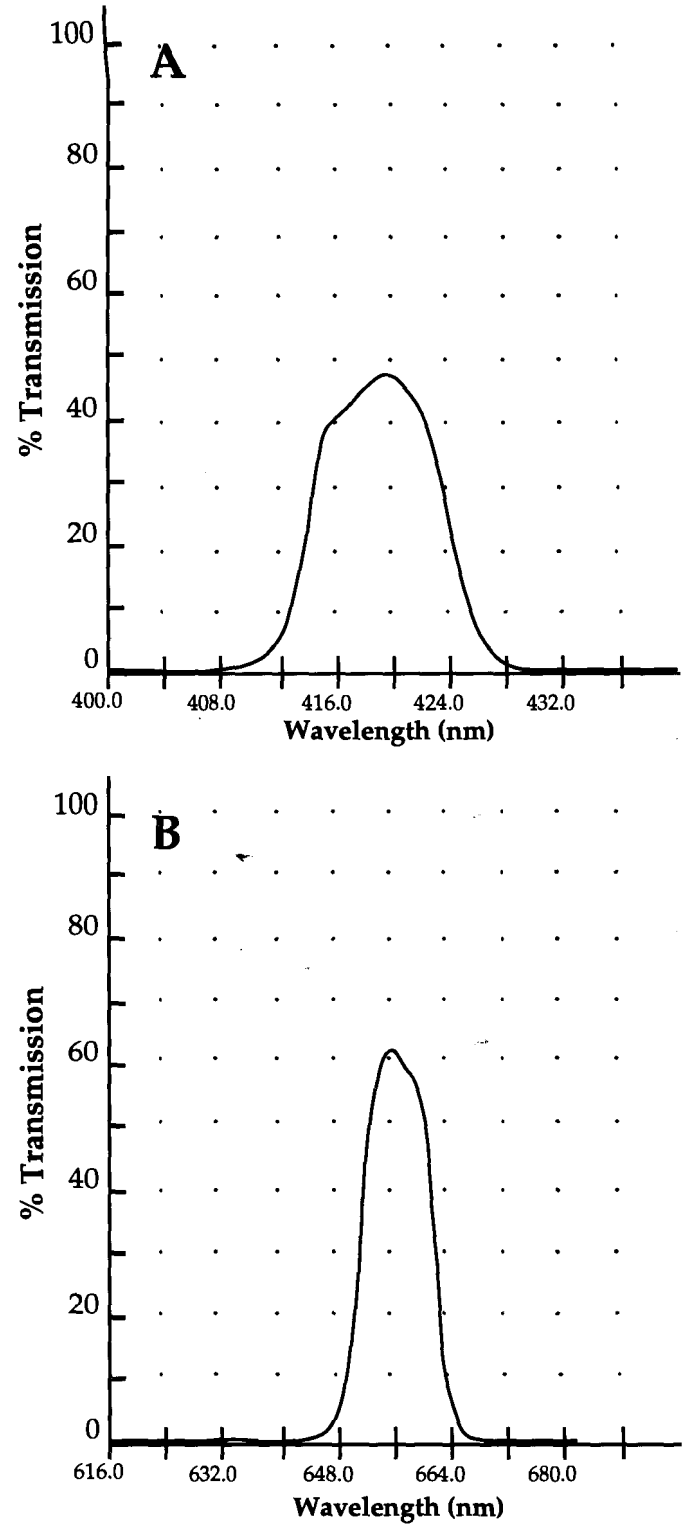


Figure 5.2.2. Light transmission of the interference filters, measured by spectrophotometry for **A.** 420 nm filter and **B.** 656 nm filter. The peak transmission of both filters was as reported by the manufacturers and the spread of transmission was approximately 20 nm in both cases.

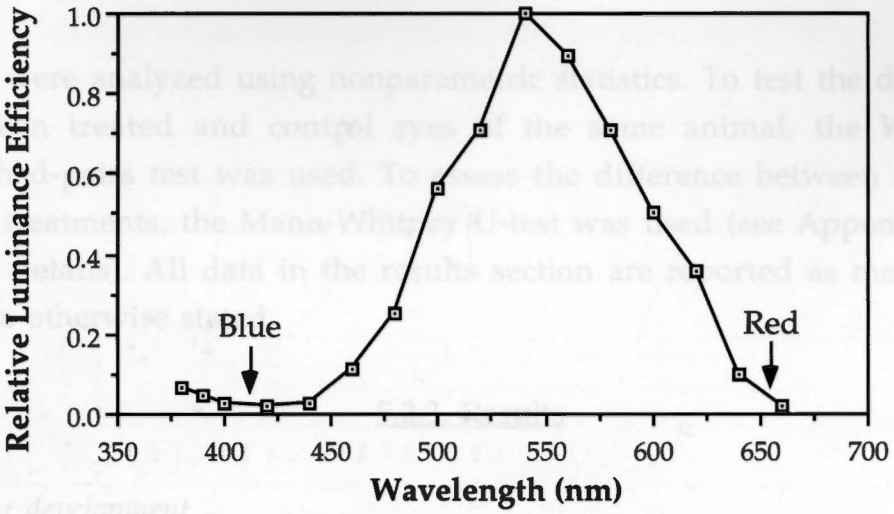


Figure 5.2.3. Relative luminous efficiency curve for the chick, adapted from Schaeffel *et al.* (1991).

Measurements

Occluders were removed after one week of occlusion and corneal power, refractive errors and axial ocular dimensions measured. These measurements were then repeated at weekly intervals for 6 weeks. All measurements were performed in dim light. Infrared-video-photokeratometry (Schaeffel and Howland, 1987) was used to measure corneal curvature; A-scan ultrasonography (Wallman and Adams, 1987) was used to measure the anterior chamber depth (ACD), axial lens thickness (ALT) and vitreous chamber depth (VCD) and axial length (AL) determined from these values. Static retinoscopy (noncycloplegic) was used to determine refractive errors. Measurements of refraction and axial ocular dimensions were made under halothane anaesthesia; corneal curvature was measured under ketamine/Rhompun anaesthesia. Chicks were sacrificed with an overdose of sodium pentobarbitone at 6 weeks of age and external ocular dimensions, i.e. axial length and equatorial diameter, measured with digital calipers (see Appendix I for more details).

To study accommodative ability, infrared-video-photorefraction (Schaeffel *et al.*, 1987) was carried out on chicks under their usual lighting conditions at 3 weeks of age (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and control eyes of the same animal, the Wilcoxon matched-pairs test was used. To assess the difference between different light treatments, the Mann-Whitney U-test was used (see Appendix I for more details). All data in the results section are reported as mean ± SD unless otherwise stated.

5.2.3. Results

Ocular development

The different light conditions did not appear to affect the growth of control eyes (see Appendix II, Tables AII.5.2 for treated and control eye data). There was a progressive increase in ACD with age, from approximately 1.2 mm at week 1 to 2.0 mm at week 6 (Fig. 5.2.5). At no time was the ACD of control eyes significantly different for chicks reared under different lighting conditions. Similarly, ALT increased with age from approximately 2.05 mm at week 1 to 2.9 mm by week 6 (Fig. 5.2.5). Again, there was no significant difference in ALT for the different lighting conditions at any age. VCD increased from approximately 5.4 mm at week 1 to 7.6 mm at week 6 (Fig. 5.2.5), while AL increased from 8.6 mm to 12.5 mm during the same period (Fig. 5.2.4). There was no significant difference in the VCD or AL of control eyes of chicks reared under the different lighting conditions at any age. Corneal power decreased with age as the anterior segment grew, values of approximately 112 D and 79 D were recorded at weeks 1 and 6 respectively (Fig. 5.2.8). Similarly, there was no differential effect of the different lighting conditions on corneal power.

The refractions of control eyes of chicks varied from low hyperopia to low myopia and thus could be broadly described as emmetropic (Fig. 5.3.4). Only at week 6 was there a significant difference in the refraction of chicks reared under the different light conditions (Table 5.2.1). Control eyes of chicks reared under red light were on average +0.80±0.40 D hyperopic compared with low myopia –0.29±0.40 D for blue light, giving a mean difference of 1.1 D between the groups ($P < 0.05$, MWUT). Although the VCD of the blue-light-reared chicks was 0.18±0.26 mm greater than that of the red-light-reared chicks this difference was not significant; a

similar trend was observed for AL. These difference are consistent with those observed for treated recovery eyes at the same age.

Table 5.2.1. Ocular parameters of open (control) eyes of chicks reared under monochromatic light at the last measurement point week 6 (mean ± SD, n = 7, 7, 8).

Ocular parameter	Treatment group		
	Blue (420 nm)	White (broad spectrum)	Red (656 nm)
Refraction (D)	−0.29±0.4•	+0.02±0.4	+0.80±0.4
Corneal power (D)	78.7±1.5	78.8±1.5	78.8±1.5
Anterior chamber depth (mm)	1.95±0.14	2.02±0.09	1.94±0.1
Axial lens thickness (mm)	2.95±0.02	2.92±0.04	2.90±0.03
Vitreous chamber depth (mm)	7.69±0.21	7.54±0.19	7.51±0.26
Axial length (mm)	12.60±0.26	12.48±0.23	12.45±0.17

Differences between red and blue groups significant at • $P < 0.05$ •• $P < 0.01$, ••• $P < 0.005$; Mann-Whitney U-test (two-tailed).

Effect of 1 week of occlusion

On eye opening at day 8, after 1 week of occlusion, those chicks reared under red light (red group) were much less myopic than those reared under either white (white group) or blue light (blue group; interocular differences Table 5.2.2; see Appendix II, Tables AII.5.2 for treated and control eye data). One week of occlusion produced a mean refractive shift of −8.02±6.2 D and −7.23±4.4 D in chicks reared under white and blue light respectively, but for those reared under red light, the mean refractive error was only −2.28±4.3 D. The differences between groups was significant when the red light group was compared with either the white or blue light treatment groups ($P < 0.01$, MWUT, both groups).

The above difference in the degree of form-deprivation myopia was due to the VCD and hence AL not increasing to the same degree in those chicks occluded under red light compared with those occluded under either white or blue light (Table 5.2.2). The mean (± SD) increase in vitreous chamber growth relative to open control eyes in chicks reared under red light was 0.19±0.12 mm; this compared with equivalent values of 0.29±0.12 mm and 0.22±0.14 mm for those reared under blue and white light respectively. This difference in vitreous chamber growth was significant when chicks reared under red and blue light were compared ($P < 0.05$, MWUT), but was not significant when chicks reared under red light were compared to those reared under white light. Corresponding mean increases in axial growth were 0.18±0.12 mm, 0.25±0.17 mm and 0.23±0.18 mm for red, blue and white light respectively (Table 5.2.2). Although the same trend as for VCD was seen, differences in the axial changes between the groups were not statistically significant.

Table 5.2.2. Differences in ocular parameters between treated (occluded 1 week) and open (control) eyes of chicks reared under monochromatic light (mean ± SD, n = 7, 7, 8).

Ocular parameter	Treatment group		
	Blue (420 nm)	**White (broad spectrum)**	**Red (656 nm)**
Δ Refraction (D)	–7.23±4.4••	–8.02±6.2**	–2.28±4.3**••
Δ Corneal power (D)	+1.59±1.9	+0.43±1.2*	+1.8±1.2*
Δ Anterior chamber depth (mm)	–0.04±0.06	–0.01±0.04	–0.02±0.02
Δ Axial lens thickness (mm)	–0.01±0.04	+0.01±0.02	+0.01±0.02
Δ Vitreous chamber depth (mm)	+0.29±0.12•	+0.22±0.14	+0.19±0.12•
Δ Axial length (mm)	+0.25±0.17	+0.23±0.18	+0.18±0.12

*Differences between red and white groups significant at * $P < 0.05$ ** $P < 0.01$, *** $P < 0.005$; differences between red and blue groups significant at • $P < 0.05$ •• $P < 0.01$, ••• $P < 0.005$; Mann-Whitney U-test (two-tailed).*

There was a slight decrease in ACD of occluded eyes compared with control eyes under all lighting conditions (Table 5.2.2). The anterior chamber was shallower by 0.02±0.02 mm, 0.04±0.06 mm, and 0.01±0.04 mm for red, blue and white groups respectively; however the ACD of occluded eyes was not significantly different from control eyes for any condition. ALT was unaffected by occlusion for all three lighting conditions used (Table 5.2.2).

Corneal curvatures were slightly steeper after one week of occlusion (Table 5.2.2). Corneal power increased by 1.8±1.2 D, 1.59±1.9 D, and 0.43±1.2 D for red, blue and white light respectively. Differences between chicks reared under red and white light were statistically significant ($P < 0.05$, MWUT).

Recovery from occlusion

Following occluder removal, myopia decreased under all lighting conditions (Fig 5.2.6). At 6 weeks of age, the final measurement point, the measured refractive error for treated eyes was –0.61±0.59 D for blue-light-reared chicks, +0.81±0.42 D for the red light group and +0.06±0.73 D for the white light group. Mean interocular differences in refraction were only –0.3 D, 0 D and +0.04 D for the blue, red and white treatment groups respectively; the refraction of recovery eyes was not significantly different from control eyes for any of the groups. These data indicate that the end refractions of treated eyes of chicks reared under blue light were more myopic than for those reared under red light; this picture is similar to that seen immediately after occluders were removed when the latter group also showed less myopia and also to the refractive errors of control eyes at week 6.

A comparison of refractive shifts of treated and control eyes revealed that during the second week, treated eyes underwent a large hyperopic shift, where as for control eyes there was a low myopic shift for all groups (Fig 5.2.9). Reflecting the magnitude of myopia produced by occlusion, hyperopic shifts during the second week were greatest for blue (+5.4±1.6 D) and white (+4.7±2.2 D) reared chicks and least for the red light group (+1.3±1.7 D). The changes in refraction during the third and subsequent weeks were similar for treated and control eyes of all treatment groups.

In parallel with the decrease in myopia during the second week there was a slowing of vitreous chamber growth in treated eyes; control eyes were unaffected (Fig. 5.2.7). For blue-light-reared chicks, vitreous

chambers of control eyes grew 0.41±0.08 mm over this period while treated eyes only grew 0.21±0.15 mm (Fig. 5.2.9). Comparable figures for the red light group were 0.66±0.16 mm and 0.51±0.25 mm, and for the white light group were 0.59±0.16 mm and 0.43±0.29 mm. During the third week, growth of the vitreous chamber of treated and control eyes was similar for all groups. Unlike vitreous chamber growth, anterior chamber growth during the second week was similar for treated and control eyes.

The ACD was only sightly shallower following two weeks form deprivation and while the ACD of treated eyes normalized over the recovery period, anterior chamber growth for most groups was not significantly less than normal. The only significant difference for anterior chamber growth occurred during week 3, where the anterior chamber of control eyes grew less than treated eyes for blue-light-reared chicks. Similarly the slight corneal steepening observed with form deprivation, normalized during the first week of restoration of vision for all groups.

External ocular dimensions

External eye dimensions were recorded at the end of the study. Treated eyes tended to be longer than those of control eyes for all light conditions (Fig. 5.2.10). This difference was significant for those chicks reared under blue light ($P < 0.05$, WSRT) and was reflected in the fact that the axial length of control eyes of blue-light-reared chicks was statistically less than for white light ($P < 0.05$, MWUT). Axial length of the treated eyes of blue-light-reared chicks was significantly greater ($P < 0.05$, MWUT)

The above pattern was not carried over to equatorial diameter data. Equatorial diameter was largest, 17.0±0.49 mm, for those chicks reared under red light, and smallest, 16.5±0.34 mm, for those chicks reared under blue light, this difference was significant for both treated and control eyes ($P < 0.05$, MWUT).

As an alternative way of examining the data, the ratio of the axial length to the equatorial diameter was calculated as a shape index; on this scale, "flat" eyes would have low values and long eyes high values, while perfectly "round" eyes would have a value of 1. This ratio was similar for the control eyes of all groups, with the equatorial diameter being greater than the axial length; the shape of the eye was unaffected by the wavelength of light. This ratio was significantly different for the treated eyes of the blue light group compared with both the red ($P < 0.05$, MWUT) and white light groups ($P < 0.05$, MWUT); the blue-light-reared chicks had

"rounder" eyes, although they still displayed the natural asymmetry of the chick eye which is for the equatorial diameter to be larger than the axial length.

Accommodative ability

Chicks were able to accommodate on near targets under all three lighting conditions. Strong accommodative responses, as indicated by a rapid change in the photorefraction image in the direction of myopia, i.e. the reflex moving from the top to the bottom of the pupil, were seen in control eyes of chicks while shifting attention to peck at food, under all lighting conditions.

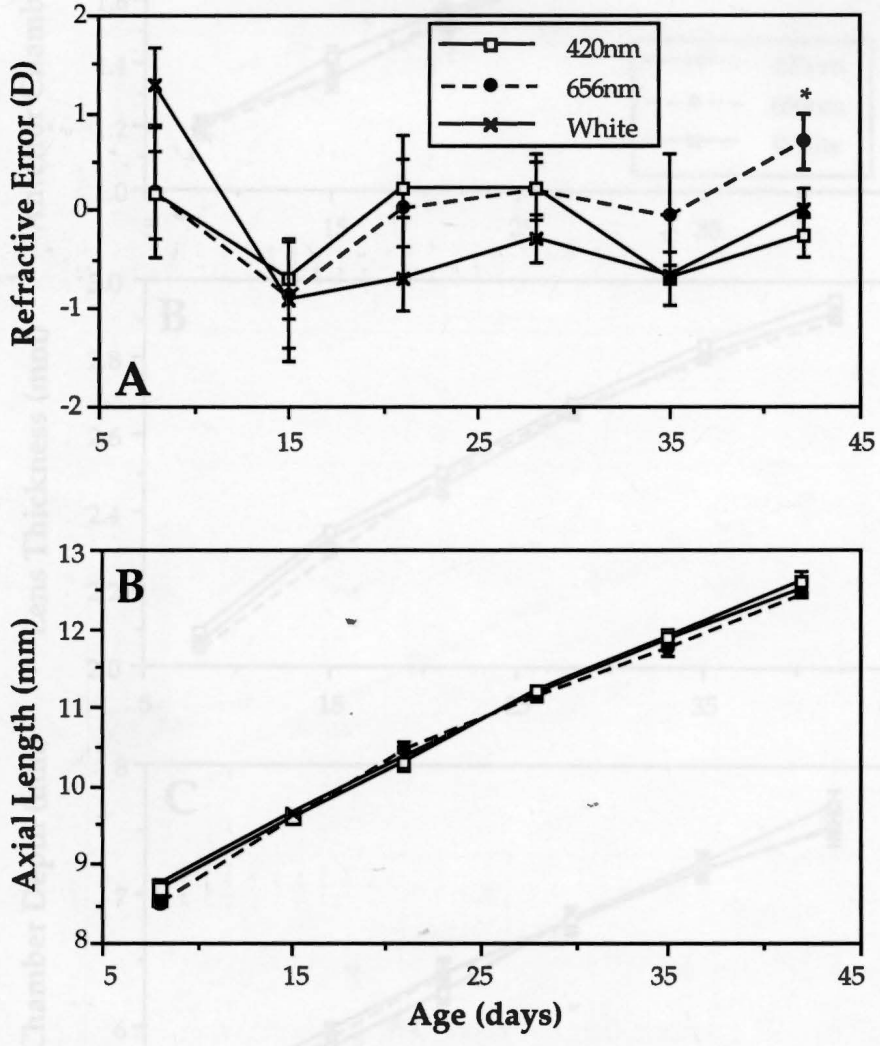


Figure 5.2.4. **A.** refraction and **B.** axial length (mean ± SE) of control eyes of chickens reared in blue (420 nm), red (656 nm) or white light. Differences between blue and red groups significant at * $P < 0.05$ **$P < 0.01$, ***$P < 0.005$, Mann-Whitney U-test (two-tailed).

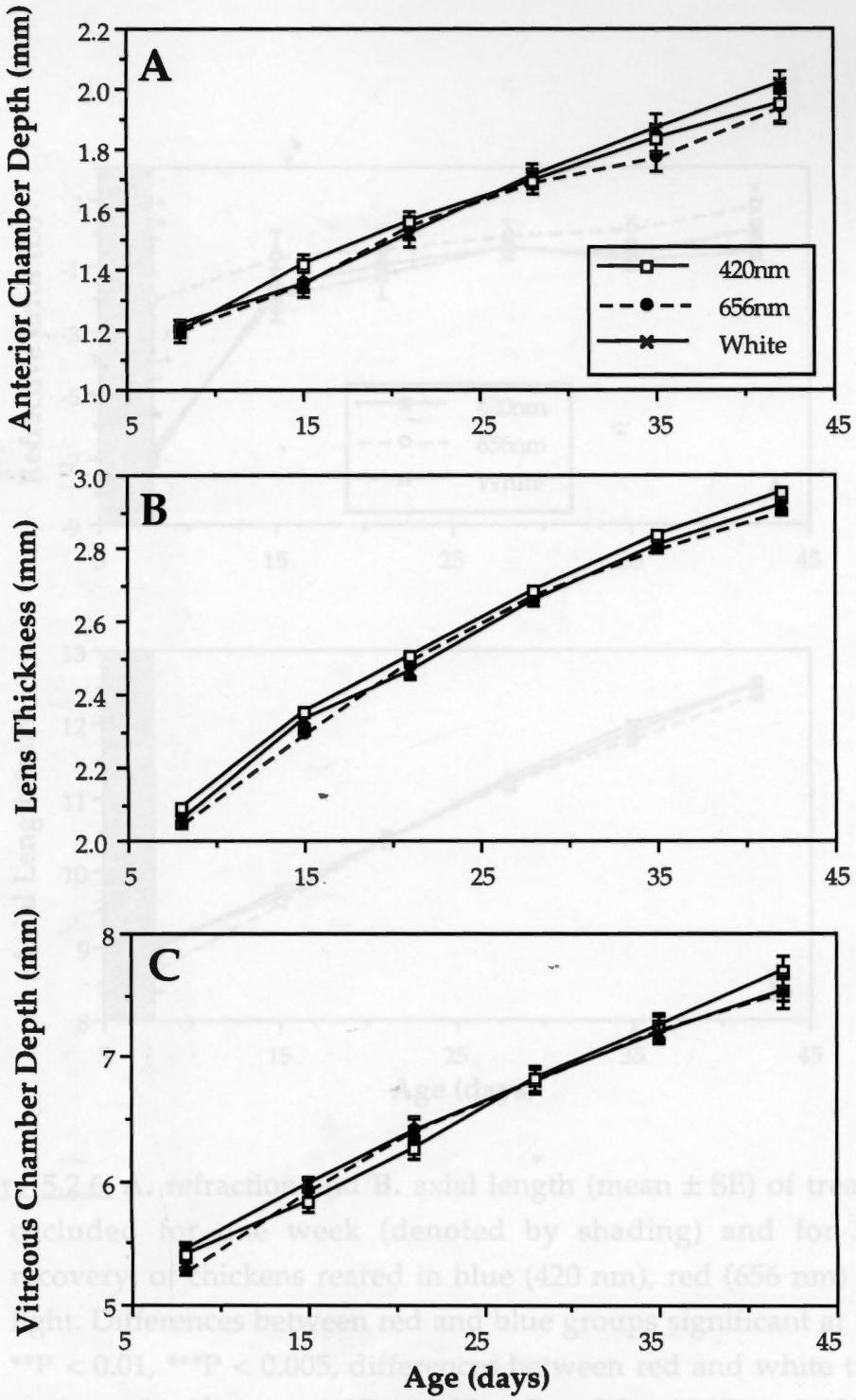


Figure 5.2.5. **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth (mean ± SE) of control eyes of chickens reared in blue (420 nm), red (656 nm) or white light. There were no significant differences between groups.

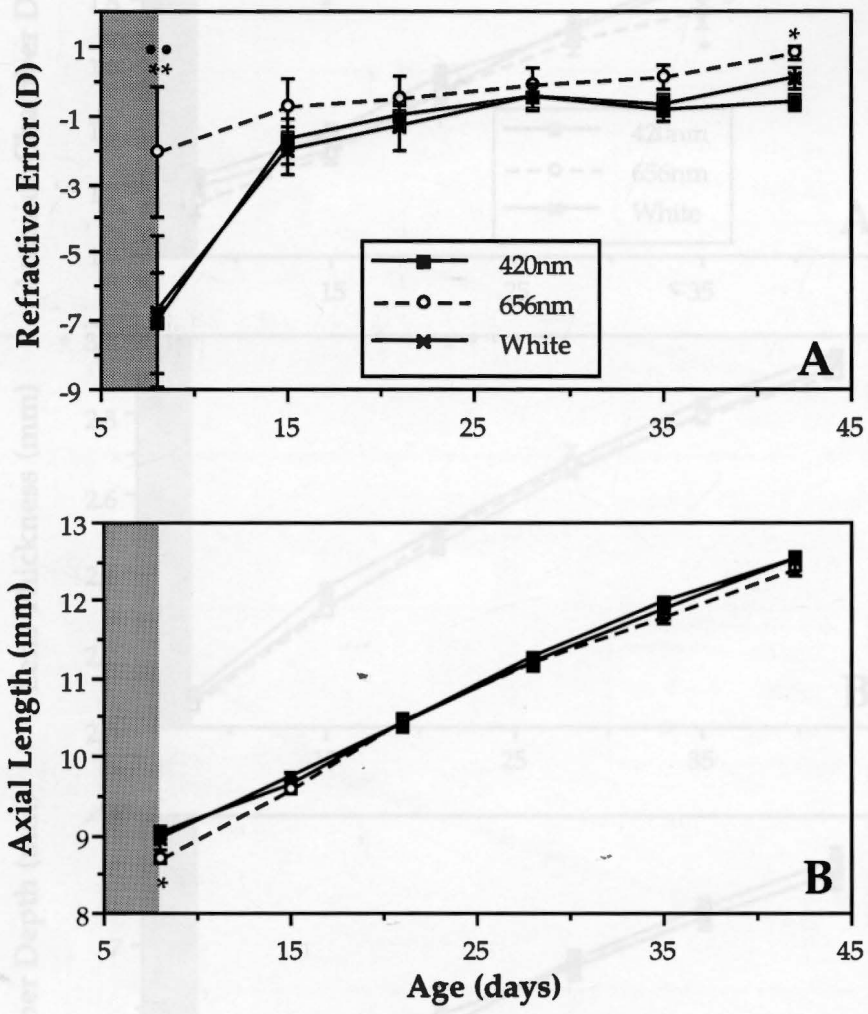


Figure 5.2.6. **A.** refraction and **B.** axial length (mean ± SE) of treated eyes occluded for one week (denoted by shading) and for 5 weeks recovery, of chickens reared in blue (420 nm), red (656 nm) or white light. Differences between red and blue groups significant at *P < 0.05 **P < 0.01, ***P < 0.005, differences between red and white treatment groups significant at •P < 0.05 ••P < 0.01, •••P < 0.005 Mann-Whitney U-test (two-tailed).

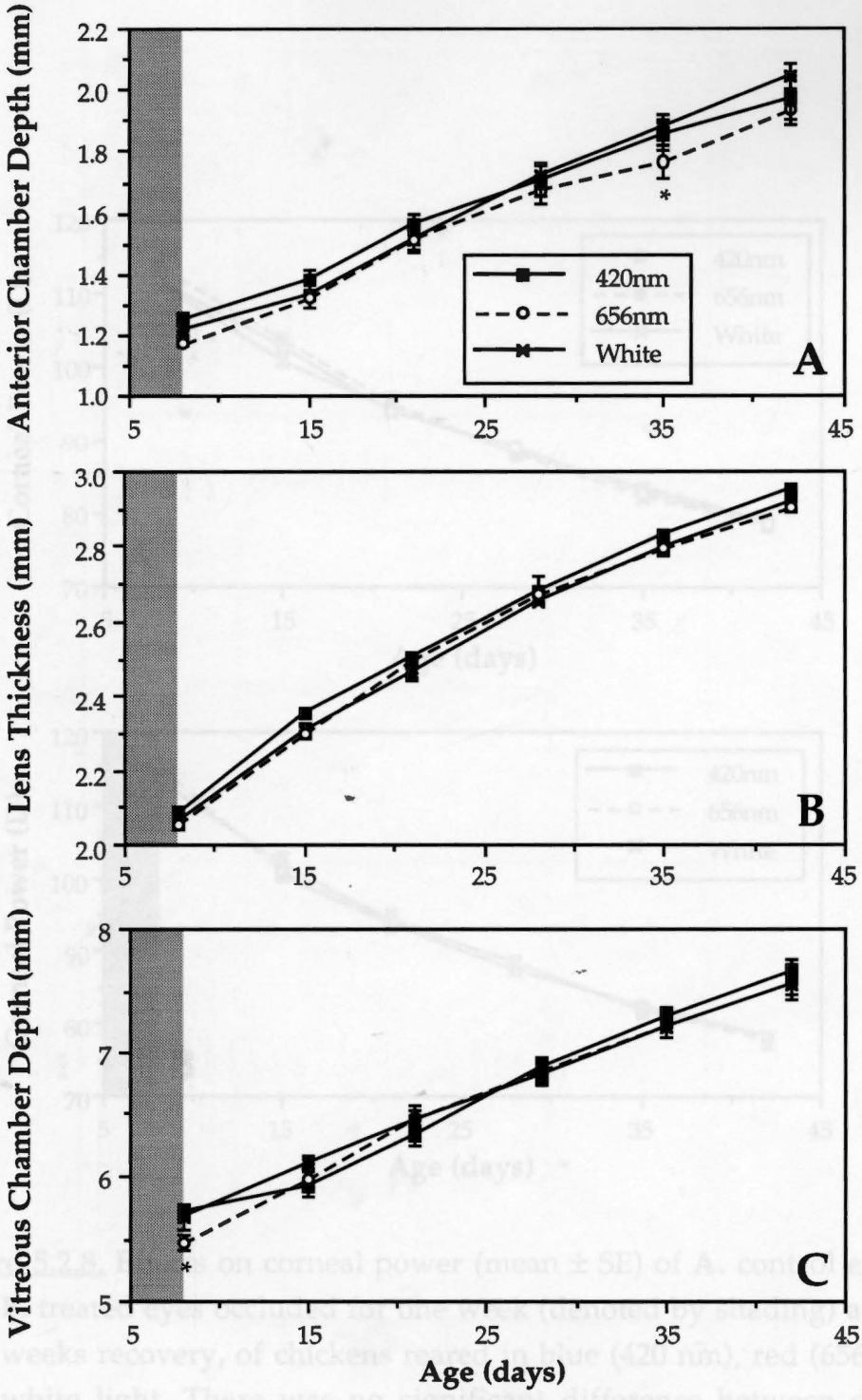


Figure 5.2.7. **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth (mean ± SE) of treated eyes, i.e. occluded for one week (denoted by shading) and for 5 weeks recovery, of chickens reared in blue (420 nm), red (656 nm) or white light. Differences between blue and red groups significant at * $P < 0.05$ **$P < 0.01$, ***$P < 0.005$, Mann-Whitney U-test (two-tailed).

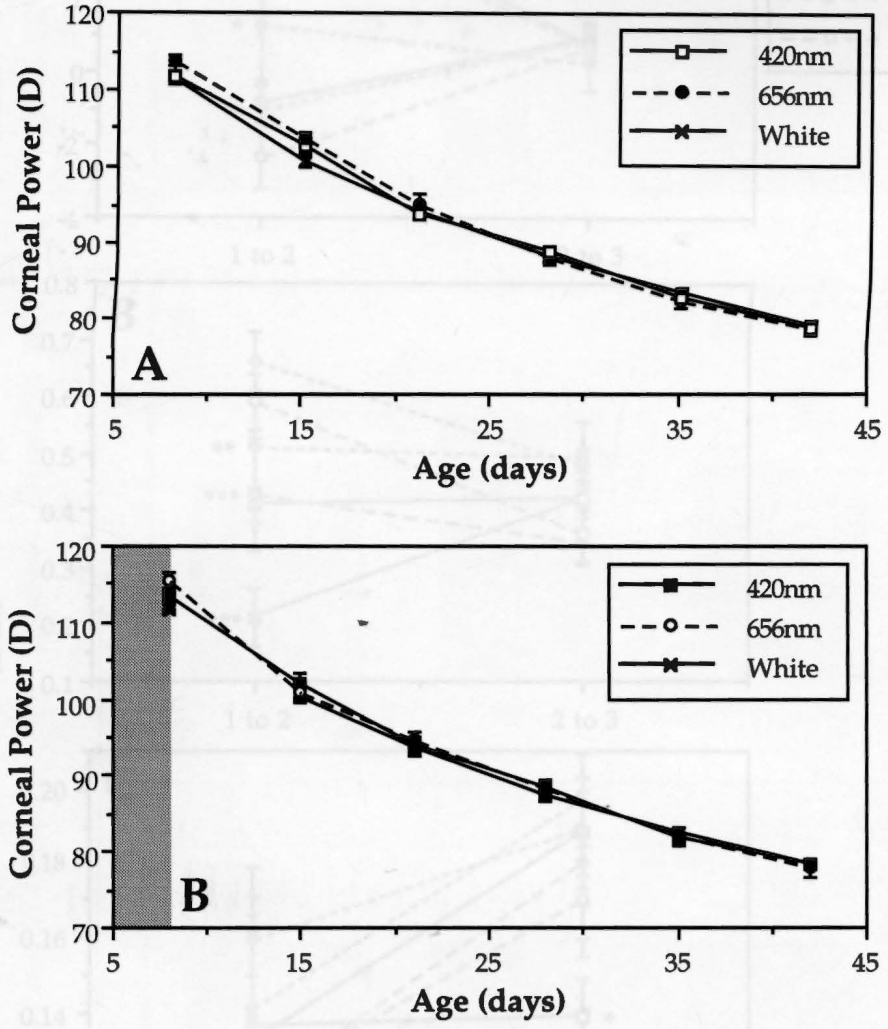


Figure 5.2.8. Effects on corneal power (mean ± SE) of **A.** control eyes and **B.** treated eyes occluded for one week (denoted by shading) and for 5 weeks recovery, of chickens reared in blue (420 nm), red (656 nm) or white light. There was no significant difference between different treatment groups.

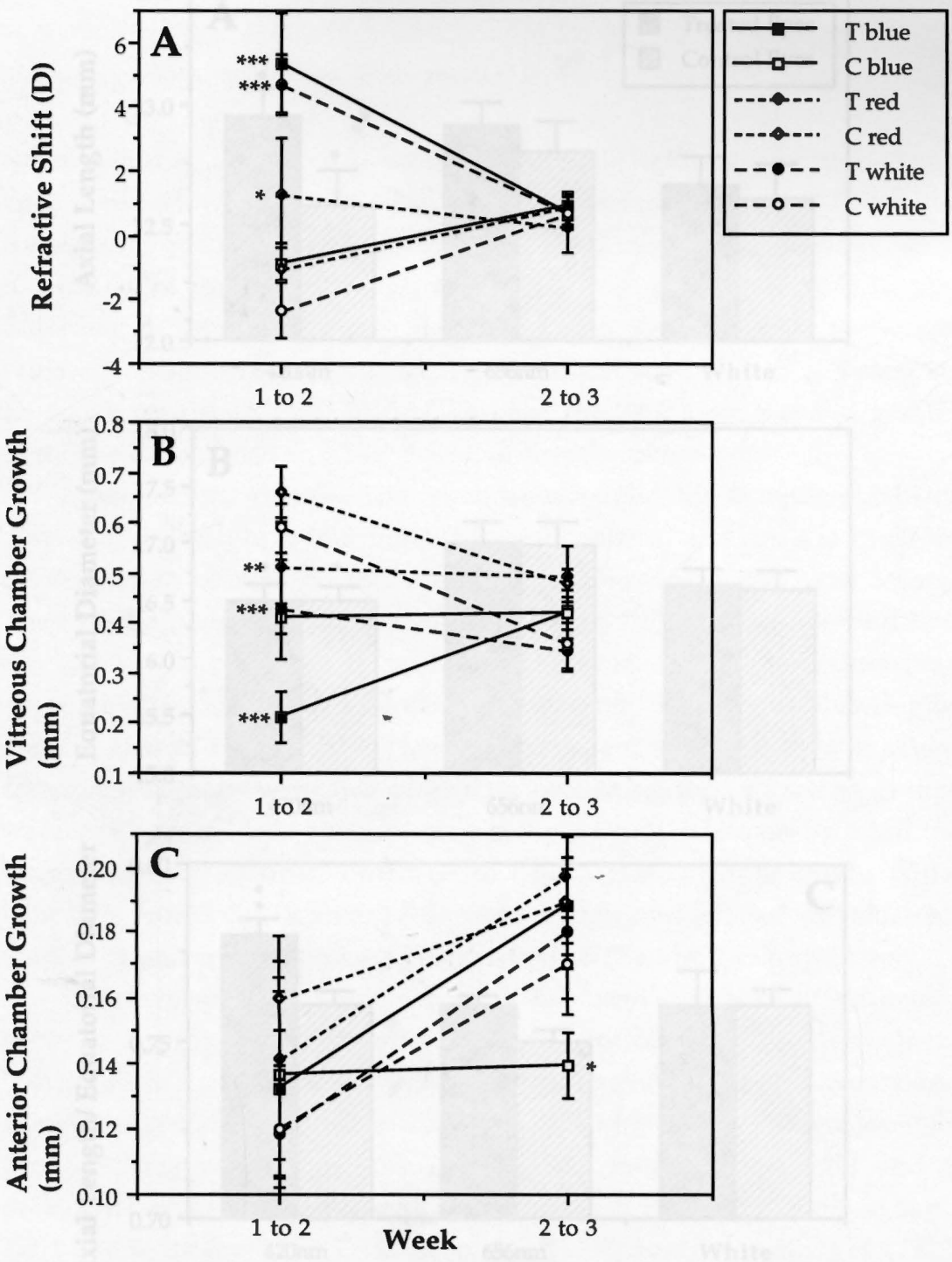


Figure 5.2.9. Comparison of treated and control eyes, **A.** refractive shift and **B.** vitreous and **C.** anterior chamber growth (mean ± SE) during the second and third weeks. Differences between treated and control eyes significant at * $P < 0.05$ **$P < 0.01$, ***$P < 0.005$, Wilcoxon matched-pairs signed-ranks test (two-tailed).

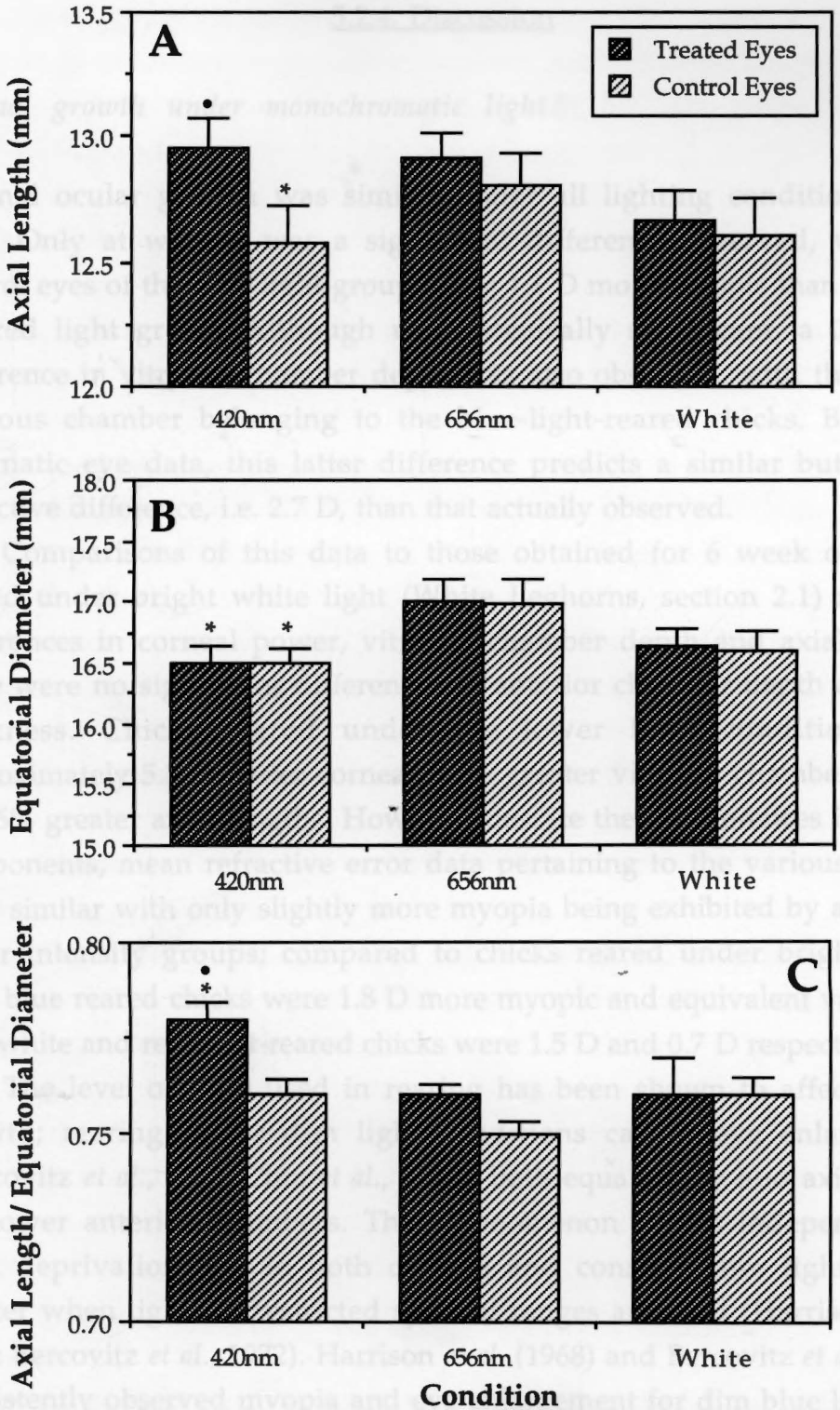


Figure 5.2.10. Effects at 6 weeks on **A.** axial length, **B.** equatorial diameter and **C.** the ratio of the axial length to the equatorial diameter (mean ± SE) of control (C) and treated (T) eyes (occluded for one week and allowed to recover), of chickens reared in blue (420 nm), red (656 nm) or white light. Difference between red and blue groups significant at *P < 0.05 **P < 0.01, ***P < 0.005, differences between red and white treatment groups significant at •P < 0.05 ••P < 0.01, •••P < 0.005 Mann-Whitney U-test (two-tailed).

5.2.4. Discussion

Normal growth under monochromatic light

Normal ocular growth was similar under all lighting conditions used here. Only at week 6 was a significant difference observed, with the control eyes of the blue light group being 1.1 D more myopic than those of the red light group. Although not statistically significant, a 0.18 mm difference in vitreous chamber depth was also observed, with the longer vitreous chamber belonging to the blue-light-reared chicks. Based on schematic eye data, this latter difference predicts a similar but greater refractive difference, i.e. 2.7 D, than that actually observed.

Comparisons of this data to those obtained for 6 week old birds reared under bright white light (White Leghorns, section 2.1) revealed differences in corneal power, vitreous chamber depth and axial length; there were no significant differences in anterior chamber depth and lens thickness. Chicks reared under the lower light conditions had approximately 5.3 D flatter corneas, 10% greater vitreous chamber depths and 6% greater axial lengths. However, despite these differences in ocular components, mean refractive error data pertaining to the various groups were similar with only slightly more myopia being exhibited by all of the lower intensity groups; compared to chicks reared under bright white light blue reared chicks were 1.8 D more myopic and equivalent values for dim white and red-light-reared chicks were 1.5 D and 0.7 D respectively.

The level of light used in rearing has been shown to affect ocular growth; rearing under dim light conditions causes eye enlargement (Bercovitz *et al.*, 1972; Chiu *et al.*, 1975), both equatorially and axially and shallower anterior chambers. This phenomenon occurs independent of form deprivation and in both diurnal and constant dim light and is greater when lights of restricted spectral ranges are used (Harrison *et al.*, 1968; Bercovitz *et al.*, 1972). Harrison *et al.* (1968) and Bercovitz *et al.* (1972) consistently observed myopia and eye enlargement for dim blue light and small hyperopic refractive errors for dim white light (+0.04±0.04 D). However, the magnitude of myopia produced by rearing under blue light in this study (–0.29±0.4 D) was much less than that reported by Harrison *et al.* (1968; –4.25 D to –17.75 D). The fact that no dim light anterior chamber effect was seen in this study would suggest that the light intensity used here was at the threshold for dim light effects. No explanation as to why

low intensity, restricted spectral light but not dim white light should cause high myopia was given by Harrison *et al.* (1968). Chiu *et al.* (1975) and Lauber and Kinnear (1979) found that diurnal dim white light (less than 0.3 lux) induced considerable eye enlargement.

Accommodation effects

Chicks could accommodate on near targets equally well under all lighting conditions, i.e. red, blue and white, ruling out differences in accommodation under the different lighting conditions as being somehow linked with the differences in final refractions. This observation suggests that chromatic aberration is not a fundamental cue for the accommodation system in chicks. Fincham (1951) reported that 40% of his human subjects responded normally to an accommodative target presented under monochromatic light. Presumably these subjects, like the chicks, are able to use alternative defocus cues. However unlike the results for the chick, some human subjects appear to depend on chromatic information for accurate accommodation.

Form deprivation under monochromatic conditions

Chicks reared under red light were not as susceptible to form-deprivation myopia as those reared under either blue or white light; the magnitude of form-deprivation myopia was much less in chicks reared under red light compared with the other lighting conditions. In an attempt to clarify the relative contributions of the various dimensional changes to the observed refractive error, predictions of changes in refraction based on measured changes in the ocular parameters and the schematic eye of Schaeffel and Howland (1988a) were made (Table 5.2.3). In all cases, predicted changes in refraction differed from measured changes, although the size of the discrepancy differed between groups. The myopic shift was underestimated for both blue and white reared chicks; in the case of white light, the myopia was underestimated by 4.4 D, i.e. over 50%. In contrast, for blue light, predicted myopia was greater than that measured using retinoscopy; although consistent with the pattern for measured refractive errors, predicted myopia for blue-light-reared chicks was still the least of the three groups. These results are unlike those for occlusion under bright light conditions where predicted myopia was similar to that measured

using retinoscopy and where changes in the anterior chamber added to the calculated myopic shift rather then subtracted from it (section 3.1). The most likely reason for these discrepancies is that changes in lens curvature, which were not measured but by exclusion are the most likely reason for the discrepancies, occurred; as these data were not available this effect was not taken into account in the predictions. Axial lens thinning has been reported in dark-reared chicks (Yinon and Koslowe, 1986) but such effects were not evident in the data here.

Table 5.2.3. Predicted compared with measured changes in refraction for 1 week of constant occlusion (CO), under different light conditions (mean ± SD, n = 7, 7, 8).

	Blue	**White**	**Red**
Measured Δ RE (D)	−7.23±4.4	−8.02±6.2	−2.28±4.3
Δ RE ACD (D)	+1.2	+0.3	+0.6
Δ RE VCD (D)	−4.6	−3.5	−3.0
Measured Δ CC (D)	+1.59±1.9	+0.43±1.2	+1.8±1.2
Predicted Δ RE (D)	−5.0	−3.6	−4.2

Predicted ΔRE based on schematic eye data of Schaeffel and Howland (1988a; see Appendix I for more details).

The form-deprivation myopia responses produced by 1 week of occlusion under the 3 dim light conditions used in the current study (which are lower than those normally used), were only 20%, 60% and 66% of that produced by 5 days of constant occlusion under higher intensity white light (250 lux; section 3.1) for red, blue and white light groups respectively. The least effect was seen for the red-light-reared chicks. Also, for all treatment groups, occlusion resulted in slight anterior chamber shallowing rather than the anterior chamber deepening usually observed with occlusion (sections 3.1, 3.2). Lauber and Oishi (1987) also noted a small inhibitory effect of deprivation on anterior segment growth under low intensity light. This may be analogous to the dim light effect on anterior chamber growth as earlier documented by Lauber and Kinnear, (1979); this effect was most evident in the red light condition of the

current study. However, the associated corneal flattening usually seen with this condition (Bercovitz *et al.*, 1972) was not observed in the current work. The lack of measured corneal response may represent limitations in the measurement technique or alternately may reflect the fact that changes in the anterior chamber were very slight.

Equatorial diameters have also been reported to enlarge under dim light conditions. The equatorial diameters of treated eyes were larger in red-light-reared chicks compared with blue-light-reared chicks suggesting a greater dim light effect for blue-light-reared chicks. Wildsoet (1992) suggested that the threshold for dim light effects was between 1 and 2.5 lux. Although light levels were 5 lux to 10 lux near the top of the cage, at the floor of the cage levels were only 0.5 lux to 1 lux which is near this critical threshold; for occluded eyes, the light level reaching the retina would have been reduced further by the treatment and for this reason could have been subthreshold. In the current study, the light intensities of all three visual stimuli were equalized based on the spectral sensitivity of the chick. It would thus seem unlikely that light intensity *per se* contributed to the observed inter-group differences in refraction, although the possibility that dim light effects are wavelength dependent cannot be ruled out.

Emmetropization under monochromatic conditions

Rearing in monochromatic conditions appeared to have little effect on the emmetropization process. This result is consistent with the findings of Schaeffel and Howland (1991) and Wildsoet *et al.* (1993) where chicks emmetropized to lens-induced defocus or recovered from form-deprivation myopia regardless of the wavelength of the incident light. In the latter study, the recovery from myopia was due to the growth of the vitreous chamber of treated eyes slowing in comparison to the growth of control eyes on restoration of normal vision; this sudden slowing of growth, as opposed to resumption of normal growth, is interpreted as evidence of active emmetropization and has been documented in a number of other studies (Wallman and Adams, 1987; reviewed in Wallman, 1993). Results reported here are consistent with these trends; emmetropization from form-deprivation myopia still occurred and control eyes maintained their emmetropic state under the monochromatic light conditions used. Together these results suggests that

alternative non-chromatic cues to defocus may be used in emmetropization.

Comparison to predictions

The difference in final refractions for those chicks reared under red and blue light was opposite to that expected based on LCA. LCA for the chick eye is approximately 3.7 D (section 5.1) and on this basis it was predicted that chicks reared under red light would have a more myopic refraction than those raised under blue light by a similar amount. It was also predicted that red-light-reared chicks would have a correspondingly deeper vitreous chamber, schematic eye calculations indicating that a 0.23 mm interocular difference in vitreous chamber depth is required to produce a 3.7 D interocular difference in focus for the chick. However neither of these predictions proved correct. To the contrary the opposite was the case, i.e. chicks reared in red light were relatively more hyperopic and had slightly shallower vitreous chambers, although this difference in refraction was only significant at week 6. A possible explanation for this apparent lack of response is that inadequate time for emmetropization was given. However in another study of responses to defocus (section 4.2), using spectacle-lens-induced defocus, emmetropization was seen after only 6 days. This would tend to rule out inadequate time for emmetropization as a possible explanation for the results. Thus the results suggest that the chick eye is not sensitive to the refractive defocus of these extreme wavelengths.

Analogy with the performance characteristics of the human accommodation system is made on the basis that both accommodation and emmetropization act to reduce defocus. The role that LCA plays in guiding accommodative focus remains controversial; reports both for (Kruger and Pola, 1986; reviewed in Kruger *et al.*, 1993) and against (Switkes *et al.*, 1990; Bobier *et al.*, 1992) LCA being used as a defocus cue for the accommodation system have been published. However, it has been recently shown that, for humans, static accommodation is insensitive to wavelength. Thus, when the spectral composition of the target is altered, there is no corresponding change in accommodation (Bobier *et al.*, 1992). Similarly, human studies report almost equal levels of accommodation when viewing varying chromatic stimuli at the same distance, even though theoretically greater accommodation is required to focus

chromatic targets of long wavelengths (Lovasik and Kergoat, 1988). This is consistent with the results obtained here which indicate that the chick eye does not emmetropize to the refractive difference of different wavelengths of light. One could argue that, given the eye by definition is sensitive to all colours in the visible spectrum and that the normal visual environment is polychromatic, emmetropization to a wavelength at an extreme of the visible spectrum would not be advantageous and indeed would be very inappropriate. As different wavelength visual stimulation was experienced different optimal focus conditions would be sought resulting in the emmetropization system being in a constant state of flux. A possible solution to this problem would be to have the relative contributions of the different wavelengths to the "emmetropization signal" weighted according to the position in the spectrum, with wavelengths at the peak of the spectral sensitivity curve being most heavily weighted, i.e. most strongly affecting emmetropization.

Another example not involving accommodation where the effects of chromatic aberration are reduced but for a different reason involves the human eye restricting the spectral composition of light for fine visual detail. Due to the absence of blue-sensitive cones in the central fovea and the presence of macular yellow pigment which absorbs blue and violet light, the human fovea only uses approximately 500 nm to 700 nm for resolving fine detail (reviewed in Knowles, 1982).

Alternative theory: level of retinal stimulation

While results indicate that chick eyes do not emmetropize to specific wavelengths, there were subtle differences between monochromatic treatment groups with respect to the final refractions for both control and recovery eyes. This warrants further consideration. Chicks reared in blue light were 1.1 D to 1.4 D more myopic than those reared under red light conditions, with refractions of white-light-reared chicks falling between the two coloured light conditions.

In offering an explanation of these differences, one needs to examine the chick retina and the possible roles of the various cone subtypes. The chick retina contains five cone types including a UV photoreceptor and 4 visible light detectors, i.e. P506, P533, P569 and P606 cone subtypes (Bowmaker and Knowles, 1977). While the sensitivity of the latter group merge to a common peak and cannot usually be separately stimulated by

restricted wavelength light, due to the relatively low light intensity used in the current work, it is possible to argue that the 420 nm light used would have mainly stimulated the P506 cones and likewise, the 656 nm light would have mainly stimulated the P606 cones.

Could the difference in final refractions observed be due to this differential photoreceptor stimulation? Under normal white light conditions, greater activity of "short wavelength pathways" compared with "long wavelength pathways" would occur if an eye were relatively hyperopic; the converse would be true if an eye were relatively myopic. It is possible that the lighting conditions used here simulated these "defocus conditions". Furthermore, if emmetropization works to eliminate such errors then one would predict the relative myopia seen in chicks reared in blue light and the relative hyperopia present in those reared in red light.

A weakness of this model is that it does not explain the lower than predicted response, i.e. 1.4 D compared with 3.7 D. Although the reduced response could theoretically reflect, in part, depth-of-focus effects in the chick eye, this explanation seems inadequate given that other data presented in this thesis indicates that chicks emmetropize to extremely low levels of spectacle defocus (section 3.3). Other factors, e.g. a wavelength weighting system (see previous page), might operate to reduce the influence of extreme wavelengths on the emmetropization process. Although the red and blue lights were matched as closely as possible in terms of visible brightness for the chick eye, the red light condition was slightly brighter (0.5 lux) then the blue. As dim light rearing results in hyperopia (Lauber and Kinnear, 1979) this effect, if present here, would have induced a refractive difference opposite to that actually observed. This could however account for some of the discrepancy between the predicted and observed difference in the results for the different coloured light treatments.

5.2.5. Conclusions

Rearing in monochromatic conditions had little effect on the emmetropization process in the chick, indicating that chromatic aberration *per se* is not a fundamental cue to the emmetropization process. This suggests that alternative "non-chromatic cues" to defocus may be used in emmetropization. Also there was no evidence that emmetropization was able to eliminate chromatic defocus associated with LCA of the chick eye; this further suggests that emmetropization is insensitive to the difference in focus of wavelengths at the spectral extremes.

5.3. The Ability of Monochromatic Light to Reduce Occlusion Induced Myopia in Chicks

5.3.0. Summary

The ability of emmetropization to still occur under monochromatic light and white light of reduced intensity was investigated. Chicks were monocularly occluded and either: i) constantly occluded, or ii) the occlusion interrupted with a period of visual stimulation. During the latter period, chicks were exposed to their normal visual environment lit by either monochromatic red, blue or yellow light or bright or dim white light. Chicks showed emmetropizing eye growth responses when occlusion was interrupted by periods of normal vision under all lighting conditions, although some differences between the end refractions of chicks exposed to different lighting conditions were observed. These results provide further evidence for the presence of an alternative non-chromatic defocus cue or cues for emmetropization.

5.3.1. Introduction

Observations from chick studies provide strong evidence for active emmetropization: i) the significant refractive errors present at hatching rapidly decrease during normal development (Wallman *et al.*, 1981), ii) chicks show compensatory refractive adaptation to lens-induced defocus (Schaeffel *et al.*, 1988; Irving *et al.*, 1991) and iii) rapid recovery from form-deprivation myopia occurs once vision is restored (Wallman and Adams, 1987). Young chicks deprived of high quality form vision, e.g. by occlusion, become severely myopic due to excessive ocular growth (Wallman *et al.*, 1978b). This excessive axial eye growth and high myopia is prevented when occlusion is interrupted by 2 hrs of "normal vision" per day (Nickla *et al.*, 1989) and is drastically reduced by as little as 20 mins of "normal vision" per day (section 3.1). These results suggest that the emmetropization system is dysfunctional when occluders are in place and that function is restored when the occluders are removed. While the findings suggest that at least some exposure to normal vision is required for emmetropization, the nature of the visual cues that might be important remain unknown.

Fincham (1951) suggested that LCA may signal the direction of defocus in the human accommodation system, as 60% of his subjects failed to respond to, or only partially responded to, an accommodative target presented under monochromatic light. Other researchers have shown that chicks can emmetropize under monochromatic yellow light; if made myopic by form deprivation, recovery from myopia, i.e. emmetropization, still occurs (Wildsoet *et al.*, 1993). However, there may be a default eye shape; when the stimulus to abnormal growth is removed, other forces may work to return the eye from an extremely abnormal eye shape to a more normal shape, thus contributing to recovery. Also, ocular compensation for spectacle-lens-induced defocus has been reported for chicks reared under red light (665 nm, cut off filter); this emmetropization response was not seen when chicks were reared under UV light (383 nm, 24 nm bandwidth; Rohrer *et al.*, 1992). These results suggest that while UV light conditions provide inadequate information for emmetropization, other "visible" monochromatic conditions allow emmetropization.

Using an interrupted occlusion paradigm, which allows chicks to be reared in a normal environment except for a short period each day, the ability of emmetropization to occur under light of different wavelengths (656±10 nm, 589 nm and 420±10 nm) and different intensities of white light (bright and dim) was investigated. This paradigm has the advantage of inducing only negligible refractive errors, providing the emmetropization process is able to proceed and thus removes the ambiguity of the emmetropization occurring passively to reduce abnormal eye shape.

5.3.2. Methods

Animals

Day-old male White Leghorn-New Hampshire cross chicks were monocularly occluded for 10 days from hatching. Chicks were either constantly occluded (CO) or the occluder was removed for 20 min/day. During the latter period chicks were exposed to their normal environment lit by either restricted wavelength light or white light. At all other times, when the occluder was in place, chicks were reared under white light (250 lux), 12 hr/ 12 hr diurnal light/ dark cycle and food and water were provided *ad libitum*. Chicks that lost their occluders were rejected from the study.

Table 5.3.1. Characteristics of the visual stimuli presented during occluder removal.

Light condition	Peak wavelength	Light level
Blue	420±10 nm	1 to 8 lux
Yellow	589 nm	0.5 to 5 lux
Red	656±10 nm	1 to 10 lux
Dim white (DW)	Broad spectrum	0.5 to 5 lux
Bright white (BW)	Broad spectrum	250 lux

Visual stimuli

Three different wavelength monochromatic lights plus two different white light intensities were used (Table 5.3.1): blue light (420±10 nm); yellow light (low pressure sodium vapour lamp, single emission line at 589 nm); red light (656±10 nm); dim white light (DW, broad spectrum daylight fluorescent light); and bright white light (BW). Blue and red light were produced by passing white light from Kodak projectors through interference filters (Edmunds Scientific); all stray white light was blocked. The spectral characteristics of the interference filters were verified using a spectrophotometer (see section 5.2) and the spectral characteristics of the sodium vapour lamp checked with a spectroscope. As outlined in the previous section, the luminance levels of the monochromatic lights were calculated on the basis of the spectral sensitivity curve of the chick (Wortel *et al.*, 1987; Schaeffel *et al.*, 1991), to be equally bright to the chick eye. Also due to the low light levels of the monochromatic light conditions (the light level for the blue light condition was the maximum achievable), a dim white light control condition was added. The intensity of the dim white light was matched with that of the monochromatic yellow light; the latter had a single emission at 589 nm which is close to the peak of the photopic spectral sensitivity curve of the chick (Bonaventure *et al.*, 1972; Schaeffel *et al.*, 1991). All light conditions were created within one of the normal chick enclosures with other chicks, food, water and food containers being present during the period of presentation.

Measurements

Refractive errors and axial ocular dimensions were measured on days 5 and 10 under halothane anaesthesia, using retinoscopy (non-cycloplegic) and A-scan ultrasonography (Wallman and Adams, 1987) respectively. Using A-scan ultrasonography the anterior chamber depth (ACD), axial lens thickness (ALT) and vitreous chamber depth (VCD) and axial length (AL) data were obtained. Corneal curvature was measured using infra-red-video-photokeratometry (Schaeffel and Howland, 1987) under ketamine/Rhompun anaesthesia. At the end of the experiment, chicks were sacrificed with pentobarbitone, the eyes enucleated and external ocular dimensions measured with calipers (see Appendix I for more details).

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated and normal eyes of the same animal, the Wilcoxon matched-pairs signed ranks test (WSRT) was used. To assess the difference between the various "lighting" groups, the Mann-Whitney U-test (MWUT) was used; interocular differences were compared in this analysis (see Appendix I for more details). All data are reported as mean ± SD unless otherwise stated.

5.3.3. Results

Constant occlusion

Constant monocular deprivation (CO) under normal bright white light conditions induced high myopic shifts in refraction, i.e. –12.0±2.0 D at day 5, increasing to –21.5±4.5 D at day 10 (interocular differences, Table 5.3.2; treated and normal eye data, Appendix II, Tables AII.5.3). This myopia was primarily due to increased growth of the vitreous chamber relative to normal eyes, 0.50±0.08 mm and 0.84±0.09 mm, at days 5 and 10 respectively. These data also closely compare with increases of 0.54±0.12 mm and 0.96±0.15 mm in AL. Anterior chamber deepening was also seen, increasing from 0.04±0.05 mm at day 5 to 0.10±0.09 mm at day 10 and accounts for the discrepancy between increases in VCD and AL. Slight, but

not significant corneal steepening was measured at both data points. There was no effect of occlusion on measured ALT.

Table 5.3.2. Differences in ocular parameters between treated and normal eyes after 5 and 10 days of constant form deprivation (mean ± SD, n = 7, 6).

Ocular parameter	Day 5	Day 10
Δ Refraction (D)	−12.0±2.0***	−21.5±4.5***
Δ Corneal power (D)	+1.4±2.2	+1.6±1.4
Δ Anterior chamber depth (mm)	+0.04±0.05	+0.10±0.09**
Δ Lens thickness (mm)	+0.002±0.01	+0.01±0.01
Δ Vitreous chamber depth (mm)	+0.50±0.08***	+0.84±0.09***
Δ Axial length (mm)	+0.54±0.12***	+0.96±0.15***

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed ranks test.*

Effect of short daily periods of visual stimulation under white light on form-deprivation-myopia

When occlusion was interrupted by short daily periods of normal vision, under the bright white light condition, form-deprivation myopia was largely prevented (interocular differences, Table 5.3.3; treated and normal eye data, Appendix II, Tables AII.5.3). Under this light condition myopic shifts of −4.4±0.5 D, i.e. 35% of CO value, and −5.6±1.5 D, i.e. 25% of CO value, were recorded after 5 days and 10 days respectively (Fig. 5.3.1, day 5; Fig. 5.3.3, day 10). When the bright white light was replaced by one of lower intensity, form-deprivation myopia was still significantly reduced, with myopic shifts of −4.4±0.6 D (35% of CO value) and −7.7±4.0 D (35% of CO value) being observed at days 5 and 10 respectively. There was no significant difference in the preventative ability, in terms of magnitude of myopic shifts in refraction, of bright (250 lux) or dim (0.5 lux) white light.

The smaller myopic shifts seen in both the above "interrupted occlusion" groups were reflected in smaller increases in vitreous chamber growth compared with CO (Fig. 5.3.2, day 5; Fig. 5.3.4, day 10) and likewise smaller increases in axial eye growth (Fig. 5.3.1, day 5; Fig. 5.3.3, day 10). Short periods of visual stimulation under bright white light reduced vitreous chamber elongation to 0.20±0.07 mm, (40% of CO value), and 0.34±0.13 mm, (40% of CO value), and axial length changes to 0.24±0.06 mm, (45% of CO value), and 0.41±0.08 mm, (45% of CO value), at days 5 and 10 respectively. Dim white light reduced vitreous chamber and axial elongation to values comparable to those recorded for the bright white light condition at day 5, i.e. 0.23±0.07 mm (45% of CO value) and 0.47±0.19 mm (55% of CO value). At day 10, dim light was slightly, though not significantly, worse than bright light at preventing these effects of occlusion, i.e. larger growth changes of 0.46±0.08 mm (55% of CO value) and 0.49±0.23 mm (60% of CO value) were observed for VCD and AL respectively.

Occlusion-induced anterior chamber deepening was slightly decreased by interrupting occlusion with either bright or dim white light (Fig. 5.3.2, day 5; Fig. 5.3.4, day 10). Increases in the ACD were 0.03±0.03 mm (75% of CO value) and 0.02±0.03 mm (50% of CO value) at day 5 for bright and dim light treatment groups respectively, and 0.06±0.09 mm (60% of CO value) and 0.03±0.07 mm (50% of CO value) respectively at day 10. Although the dim light group showed less change in ACD, this difference between groups was not significant.

Axial lens thickness was unaffected by occlusion and there was no significant effect of the two interrupted-occlusion paradigms on lens thickness (Fig. 5.3.2, day 5; Fig. 5.3.4, day 10).

Effect of short daily periods of visual stimulation under monochromatic light on form-deprivation myopia

Monochromatic light provided adequate visual stimulation for reducing the response to form deprivation under the experimental paradigm used. Under monochromatic blue light conditions, myopic shifts of –4.8±0.9 D (40% of CO level) and –10.5±6 D (50% of CO level) were observed after 5 and 10 days treatment respectively (interocular differences, Table 5.3.3; treated and normal eye data, Appendix II, Tables AII.5.3; Fig. 5.3.1, day 5; Fig. 5.3.3, day 10). These values are similar to those for bright white light at day 5, but blue light was slightly less effective at preventing myopia at

day 10. Myopic shifts for the monochromatic yellow light treatment group were –5.2±0.6 D (45% of CO level) and –6.7±2.5 D (30% of CO level) at days 5 and 10 respectively and the equivalent values for monochromatic red light group were –6.2±1.7 D (50% of CO level) and–6.0±2.4 D (30% of CO level). The monochromatic red light condition was slightly, but not significantly, less effective than bright white light at preventing occlusion-induced changes in refraction at day 5; the values for the yellow treatment groups were similar to the bright white light at both ages.

As with visual stimulation under white light, reductions in occlusion-induced myopia for monochromatic light were reflected in smaller increases in vitreous chamber and axial eye growth. Measured vitreous chamber elongation was 0.22±0.07 mm (45% of CO level), 0.25±0.06 mm (50% of CO level), and 0.31±0.07 mm (60% of CO level) for the monochromatic blue, yellow and red light treatment groups respectively at day 5 and were 0.53±0.24 mm (65% of CO level), 0.39±0.11 mm (45% of CO level) and 0.37±0.13 mm (45% of CO level) respectively at day 10 (Fig. 5.3.2, day 5; Fig. 5.3.4, day 10). Thus, the red light condition was least effective at preventing vitreous elongation at day 5, and the blue light condition was least effective at day 10. Measured axial elongation was 0.26±0.13 mm (50% of CO level), 0.27±0.09 mm (50% of CO level), 0.36±0.07 mm (65% of CO level) for the monochromatic blue, yellow and red light treatment groups respectively at day 5 and were 0.61±0.34 mm (65% of CO level), 0.43±0.18 mm (45% of CO level) and 0.40±0.15 mm (50% of CO level), respectively at day 10. Reflecting vitreous chamber data, the red light condition was also least effective at preventing axial elongation at day 5 and the blue condition was also least effective at day 10.

When occlusion was interrupted by monochromatic light stimulation, eyes still showed the characteristic anterior chamber deepening associated with occlusion; thus differences from the CO group were slight or non-existent at day 5 (Fig. 5.3.2, day 5; Fig. 5.3.4, day 10). An increases in ACD of +0.03±0.03 mm (75% of CO level) was recorded for yellow light. Neither stimulation under red light nor blue light affected the increase in ACD due to occlusion, with values for these groups being 0.04±0.03 mm (100% of CO level) and 0.04±0.05 mm (100% of CO level), respectively. At day 10, groups exposed to either yellow or red light showed reduced effects of occlusion on the anterior chamber; measured changes were 0.04±0.09 mm (40% of CO level) and +0.03±0.09 mm respectively (30% of CO level). Again stimulation under blue light gave

results comparable to constant occlusion, with 0.09±0.09 mm deepening of the ACD (90% of CO level) being observed.

Axial lens thickness was unaffected by constant form deprivation and there was similarly no effect on the lens when occlusion was interrupted with restricted wavelength stimulation (Fig. 5.3.2, day 5; Fig. 5.3.4 day 10).

Table 5.3.3. Effect of short periods of visual stimulation on occlusion-induced form-deprivation myopia. Differences in refraction of treated and normal eyes measured at day 5 and day 10 (mean ± SD, n).

	Δ Refraction (D)	
Treatment group	**Day 5**	**Day 10**
Constant occlusion	–12.0±2.0, 7	–21.5±4.5, 6
Bright white light	–4.4±0.5, 8***	–5.6±1.5, 6***
Dim white light	–4.4±0.6, 10***	–7.7±4.0, 8***
Monochromatic blue	–4.8±0.9, 10***	–10.5±6, 8***•
Monochromatic yellow	–5.2±0.6, 10***	–6.7±2.5, 7***
Monochromatic red	–6.2±1.7, 10***•	–6.0±2.4, 6***

*Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between bright white light and restricted wavelength light groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).*

Predicted changes in refraction based on ocular parameter changes

Predicted changes in refractive error based on measured changes in anterior and vitreous chamber depths and corneal power were similar to those measured experimentally, for all "interrupted-occlusion" treatment groups, at both days 5 and 10 (Table 5.3.4). Thus, where measured refractive errors were low, so were predicted values and vice-versâ. This analysis also confirmed that vitreous chamber elongation contributed most to the myopic shift seen for all treatment groups.

Table 5.3.4. Predicted (based on ocular parameter changes) compared with measured changes in refraction for occlusion interrupted by restricted light (intensity of wavelength) stimulation, at day 5 and 10.

	Day 5					Day 10				
	Blue	**Yellow**	**Red**	**DW**	**BW**	**Blue**	**Yellow**	**Red**	**DW**	**BW**
Measured Δ RE (D)	–4.8	–5.2	–6.2	–4 4	–4.4	–10.5	–6.7	–6.0	–7.7	–5.6
Δ RE ACD (D)	–1.1	–1.0	–1.2	–0.6	–1.0	–2.5	–1.0	–0.9	–0.9	–1.8
Δ RE VCD (D)	–3.5	–4.0	–4.9	–3.6	–3.2	–8.4	–6.2	–5.6	–7.4	–5.4
Measured Δ CP (D)	+0.6	–0.1	–0.4	+0.1	–1.3	+0.4	+0.1	–1.3	–0.8	–0.7
Predicted Δ RE (D)	–5.2	–4.9	–5.7	–4.3	–2.9	–11.3	–7.3	–5.2	–7.5	–6.3

Predicted ΔRE based on schematic eye data of Schaeffel and Howland, (1988a; see Appendix I for more details).

Enucleated eye data

The external axial length of treated eyes was significantly greater than contralateral normal eyes for all treatment groups (see Appendix II, Table AII.5.3.3 for treated and normal eye data; Fig 5.3.6). The interocular difference was greatest for the constant occlusion group, i.e. 0.90±0.18 mm, and was significantly less for all interrupted-occlusion groups (45% to 55% of CO level; $P < 0.005$, MWUT all groups cf CO). There was no significant difference between the different interrupted-occlusion groups with respect to increased axial growth.

Similarly, equatorial diameters of treated eyes were greater than those of normal eyes for all groups, although the increases were less than those seen for axial growth. Again the interocular difference was greatest for the constant occlusion group, i.e. 0.40±0.18 mm, but was only significantly less for the blue, red and dim white light treatment groups. The equatorial expansion recorded for the yellow and bright white light groups was not significantly different to that observed with CO. The dim white and blue light treatment groups showed significantly less equatorial expansion than the bright white light group.

The increases in eye size as indicated by the larger axial and equatorial dimensions of treated eyes were coupled with an increase in

wet weights for all treatment groups. Again, the greatest difference was seen for CO, 0.098±0.009 g, and values were significantly less for all interrupted occlusion groups, i.e. approximately 50% of CO level.

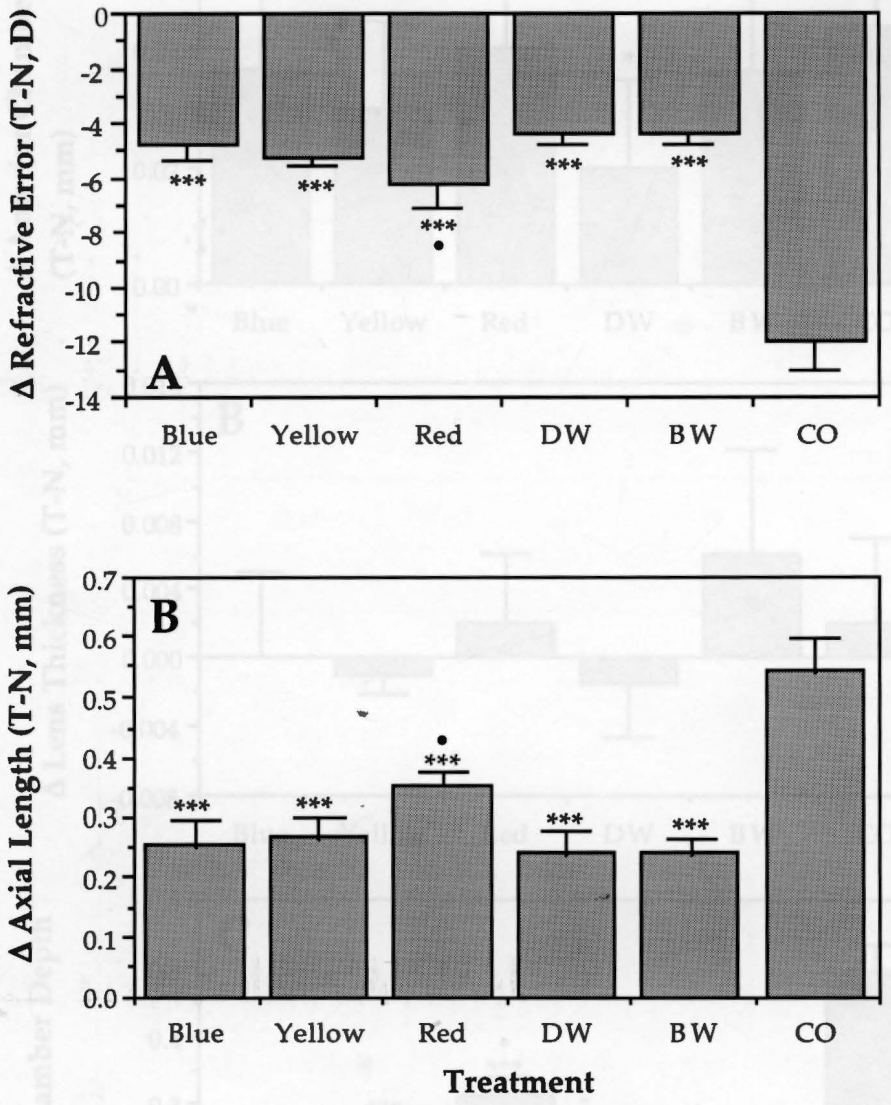


Figure 5.3.1. Differences (mean ± SE) at day 5, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light. Differences between CO and interrupted CO groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between BW and interrupted CO groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

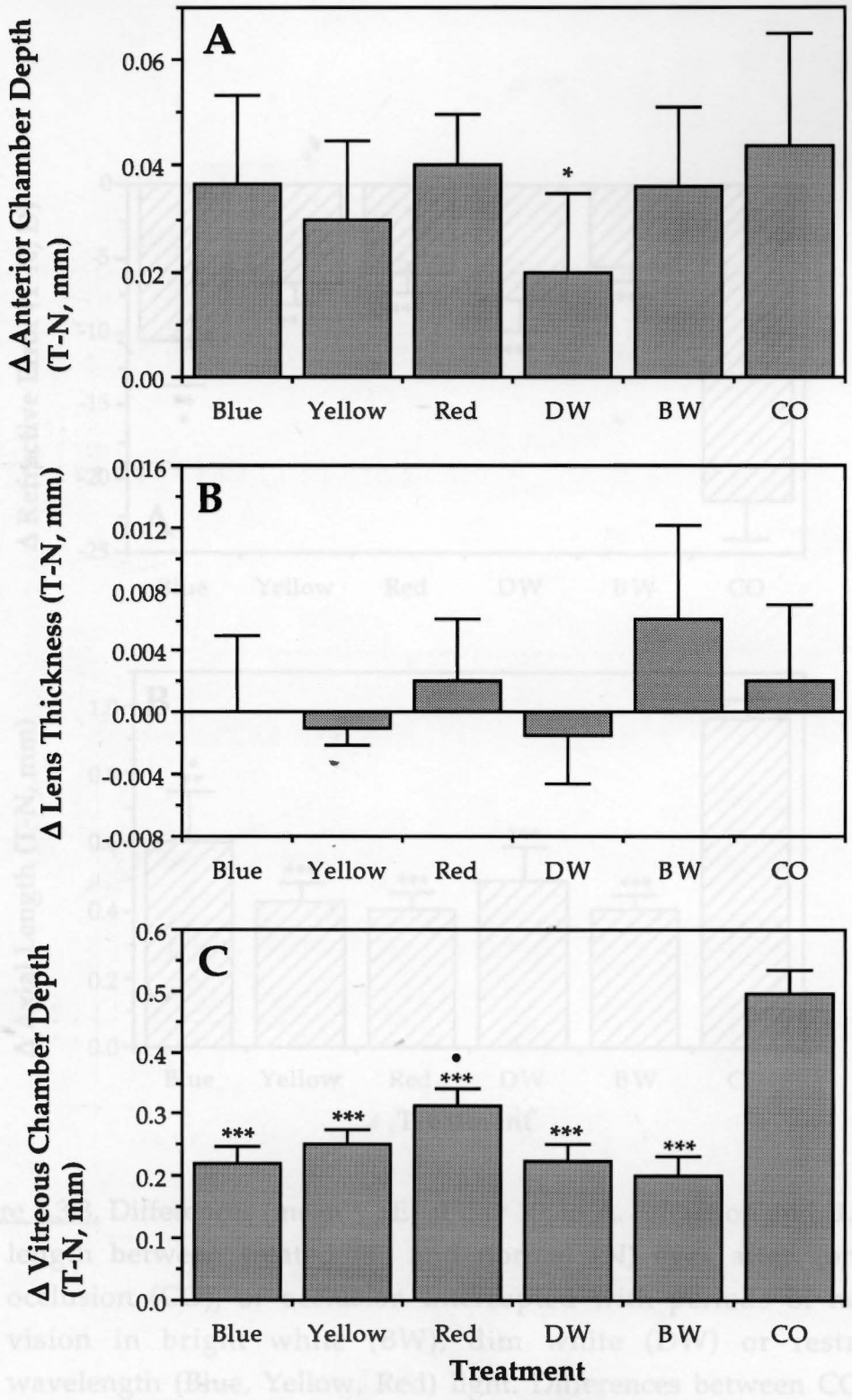


Figure 5.3.2. Differences (mean ± SE) at day 5, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light. Differences between CO and interrupted CO groups significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, differences between BW and interrupted CO groups significant at $^{\bullet}P < 0.05$, $^{\bullet\bullet}P < 0.01$, $^{\bullet\bullet\bullet}P < 0.005$, Mann-Whitney U-test (one-tailed).

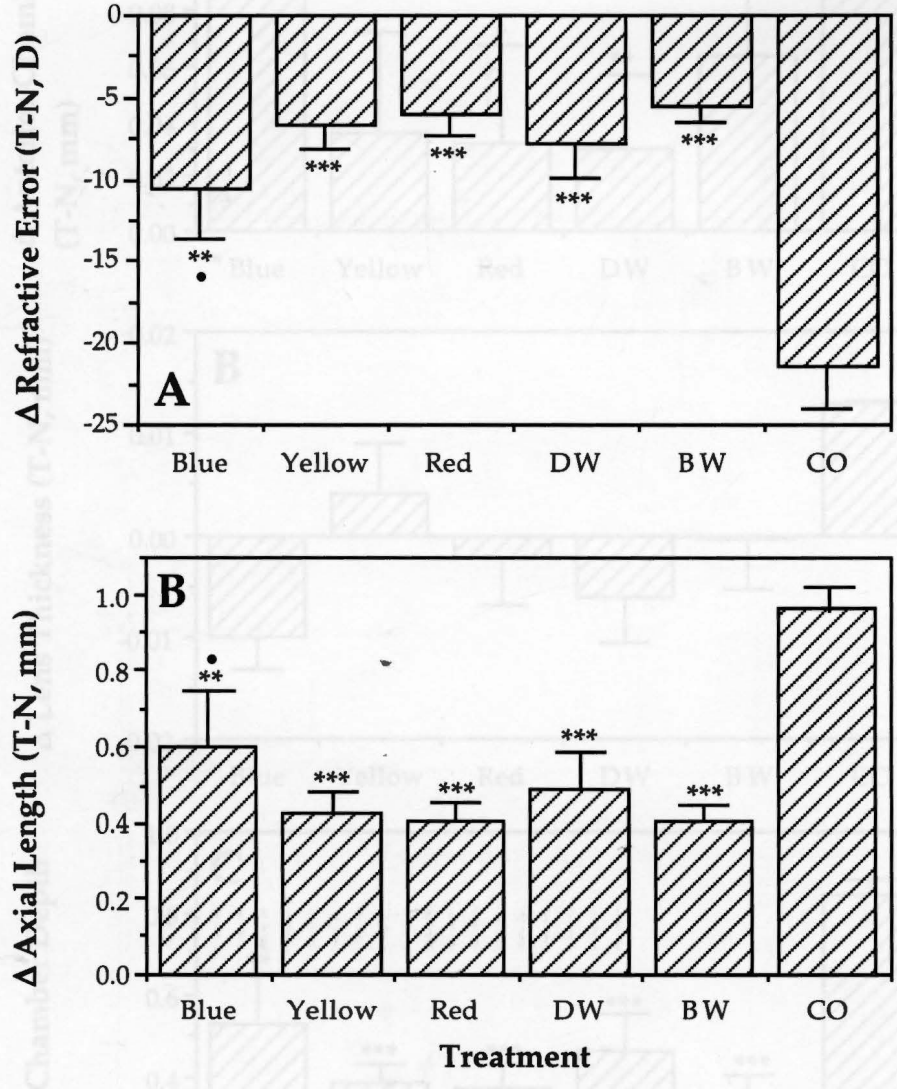


Figure 5.3.3. Differences (mean ± SE) at day 10, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light. Differences between CO and interrupted CO groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between BW and interrupted CO groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

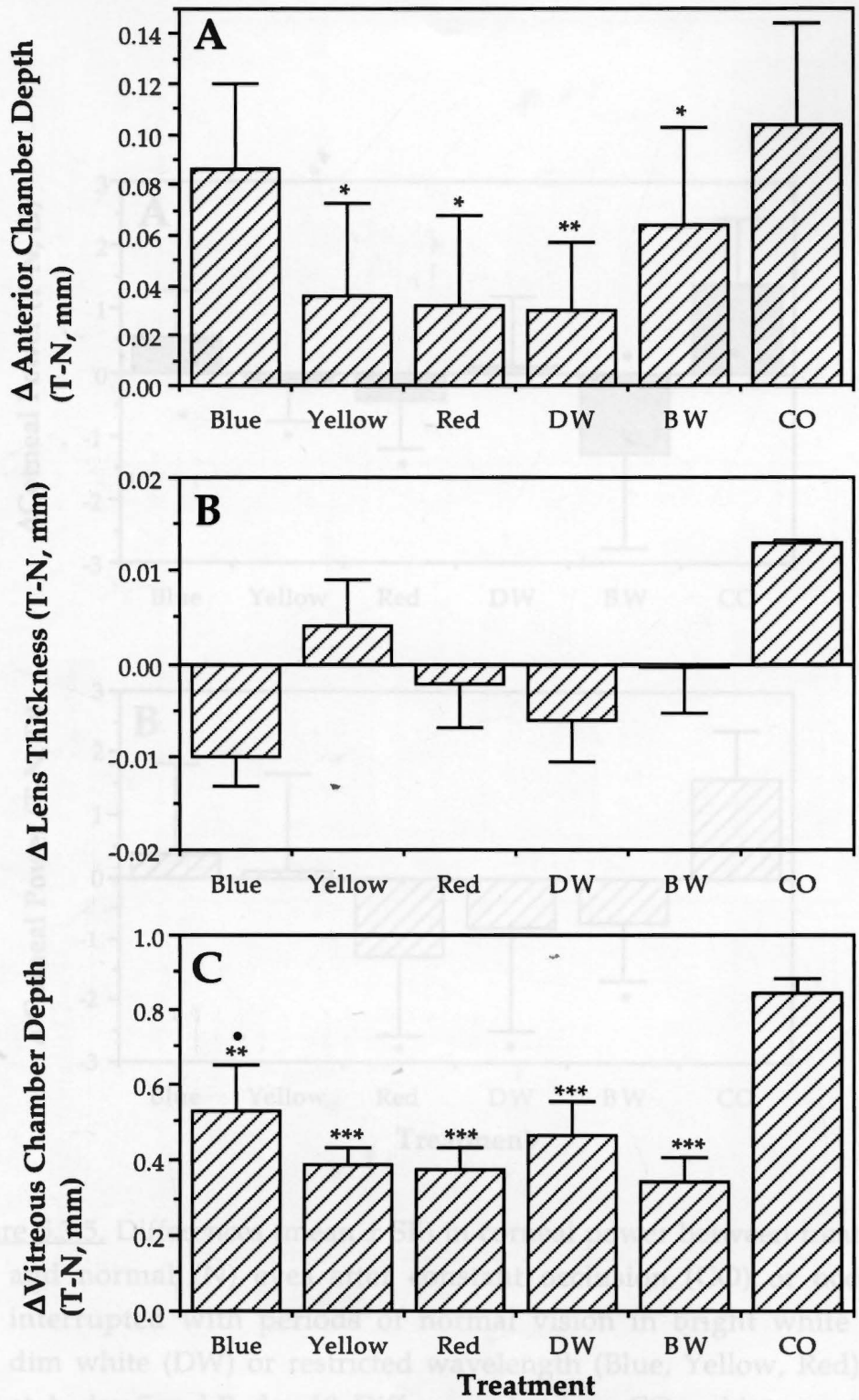


Figure 5.3.4. Differences (mean ± SE) at day 10, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light. Differences between CO and interrupted CO groups significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, differences between BW and interrupted CO groups significant at •$P < 0.05$, ••$P < 0.01$, •••$P < 0.005$, Mann-Whitney U-test (one-tailed).

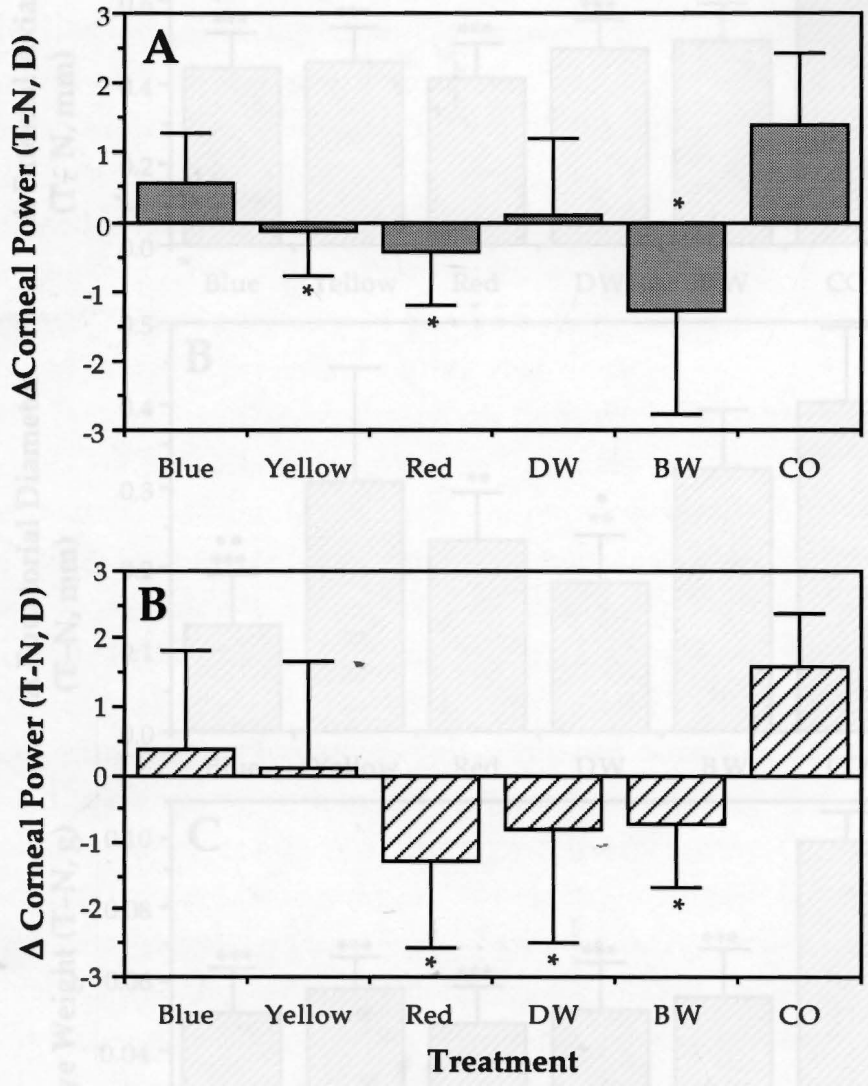


Figure 5.3.5. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes after constant occlusion (CO) or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light, at **A.** day 5 and **B.** day 10. Differences between CO and interrupted CO groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between BW and interrupted CO groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

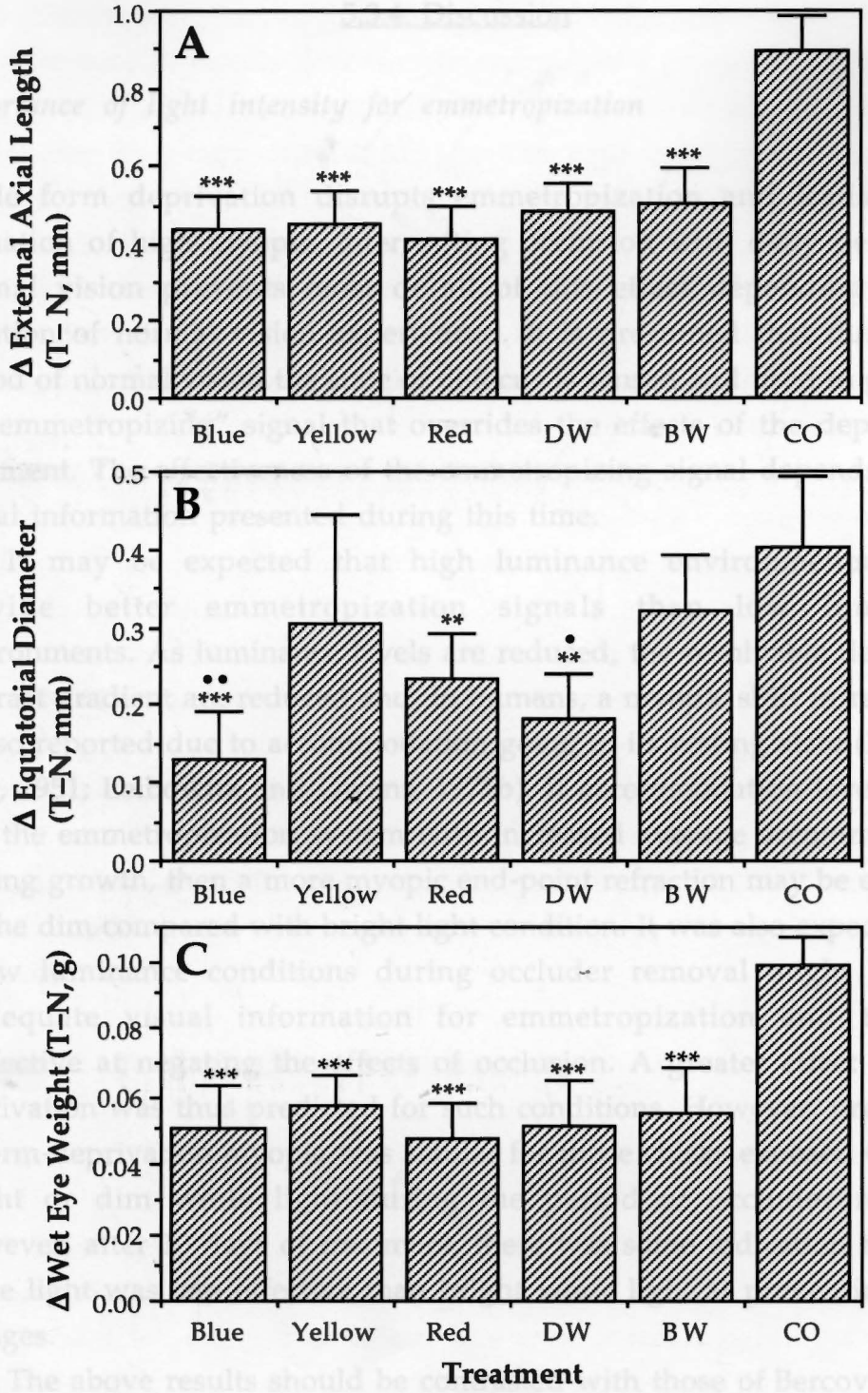


Figure 5.3.6. Differences (mean ± SE) at day 10, in **A.** external axial length, **B.** equatorial diameter and **C.** wet eye weight between treated (T) and normal (N) eyes after constant occlusion (CO), or occlusion interrupted with periods of normal vision in bright white (BW), dim white (DW) or restricted wavelength (Blue, Yellow, Red) light. Differences between CO and interrupted CO groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between BW and interrupted CO groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

5.3.4. Discussion

Importance of light intensity for emmetropization

While form deprivation disrupts emmetropization and leads to the formation of high myopia, interrupting occlusion with daily periods of normal vision prevents most or all of this effect depending on the duration of normal vision experienced. It is presumed that during the period of normal vision the state of defocus is sensed and used to generate an "emmetropizing" signal that overrides the effects of the deprivation treatment. The effectiveness of the emmetropizing signal depends on the visual information presented during this time.

It may be expected that high luminance environments would provide better emmetropization signals than low luminance environments. As luminance levels are reduced, the resolvable detail and contrast gradient are reduced and, in humans, a myopic shift in refraction is also reported due to accommodation going to its resting state (Koomen *et al.*, 1951; Leibowitz and Owens, 1975b). If accommodation levels input into the emmetropization system with increased average accommodation driving growth, then a more myopic end-point refraction may be expected for the dim compared with bright light condition. It was also expected that a low luminance conditions during occluder removal might provide inadequate visual information for emmetropization and thus be ineffective at negating the effects of occlusion. A greater effect of form deprivation was thus predicted for such conditions. However, prevention of form-deprivation myopia was similar for those chicks exposed to either bright or dim white light during the period of occluder removal. However, after 10 days of treatment, there was some indication that dim white light was less effective than bright white light at preventing these changes.

The above results should be contrasted with those of Bercovitz *et al.* (1972) and Chiu *et al.* (1975) who found, using a different experimental paradigm, that rearing under dim light conditions *per se* caused eye enlargement, both equatorially and axially. Similarly, only poor recovery from form-deprivation myopia has been reported if chicks are placed in dim light during the recovery period (Gottlieb *et al.*, 1991). These results suggest that dim light conditions are inadequate for emmetropization. However, in data presented here, the dim light condition was as nearly as

good at preventing form-deprivation myopia as the bright light condition. The difference in results lend further support to a previous proposal that there is a threshold below which dim light does not provide adequate information for emmetropization. The dim light condition of Gottlieb *et al.* (1991) was only 0.005 lux which is more than two log units less than that used in the current study.

There are also fundamental design differences between the current study and other cited studies which may be of significance. For example in the studies of Bercovitz *et al.* (1972) and Chiu *et al.* (1975) chicks were reared in dim light, while chicks were exposed only to short periods of dim light, i.e. 20 min, in the current study. Thus one reason for this difference in results may be that rearing in dim light conditions results in a damping of the diurnal variations in dopamine, melatonin and serotonin (Hamm and Menraker, 1980) thereby resulting in eye enlargement. In the current study chicks were reared in bright diurnal light, i.e. 12 hr light/dark cycle, except for a short period of dim light exposure and thus circadian rhythms would have been less affected. This interpretation is also consistent with the suggestion by Wallman (1991) that the eye enlargement caused by both constant light and constant darkness is linked to the disruption of circadian rhythms. Disruption of diurnal rhythms also appears to be linked to eye enlargement in other species (Iuvone *et al.*, 1991).

Importance of wavelength of light for emmetropization

The possibility that chromatic aberration could provide information regarding defocus for the emmetropization process has been raised by many investigators (Schaeffel and Howland, 1991; Rohrer *et al.*, 1992; Wildsoet *et al.*, 1993), although to date, studies have been limited to the chick model for emmetropization. The experimental paradigm used in the study described here is very different from that of other reported studies: it is assumed that the period of "normal vision" represents the "active defocus sensing period" and thus chicks were exposed to only short durations of monochromatic light conditions rather than constantly reared under monochromatic light as in the studies of Rohrer *et al.* (1992) and Wildsoet *et al.* (1993). However, despite these differences, results reported here also suggest that chromatic cues are not essential for the emmetropization process; reductions in form-deprivation myopia were

not significantly different for those chicks given "normal vision" under monochromatic blue, yellow, red light or white light. Furthermore, wavelengths at the extremes of the visible spectrum were as good at preventing form-deprivation myopia as those at the centre of the spectrum.

It is, of course, possible that the hypothesis that defocus cues are detected during the period of "normal vision" is invalid and that some other, yet to be determined, mechanism mediates the interrupted-occlusion paradigms. There were subtle differences between some of the treatment groups although trends were not consistent over time, e.g. at day 5, monochromatic red light was least effective at preventing occlusion-induced changes in refraction and axial length, while by day 10 the monochromatic blue light condition was least effective; at the latter time point an average 4.5 D greater residual myopia was observed in the blue light compared with red light treatment groups. Interestingly, in the study reported in section 5.2, chicks reared under blue light also showed greater myopia at the last measurement point and thus a similar explanation may apply to both sets of results. One explanation put forward in the previous section (5.2) implicated differential photoreceptor stimulation. To re-iterate, if an eye was relatively hyperopic then under normal white light conditions, greater activity of "short wavelength pathways" compared with "long wavelength pathways" would occur; the converse would be true if an eye was relatively myopic. It was proposed that the monochromatic red or blue lighting conditions could simulate these "defocus conditions" with red light being associated with myopia and blue light with hyperopia. Furthermore, if emmetropization works to eliminate such defocus errors, then one would predict the relative myopia seen in the blue light treatment group and the relative hyperopia present in the red light treatment group.

It has been suggested by analogy that, as chromatic aberration provides information regarding defocus for the accommodation system, the emmetropization process may also use chromatic aberration as a cue to defocus (Wildsoet *et al.*, 1993). There are, however, conflicting views at least for human, as to the role chromatic aberration plays in guiding the accommodation system; while studies show that longitudinal chromatic aberration may be important under some conditions and/or for some subjects, other subjects are able to accurately accommodate under monochromatic light (Fincham, 1951; reviewed in Charman and Tucker,

1978a and Kruger *et al.*, 1993). Accommodation results suggest that perhaps there are other non-chromatic cues to defocus that can be used to guide emmetropization; under normal chromatic light conditions these cues may be more important for accommodation in some subjects compared with others. This could explain the inter-subject differences in responses when chromatic cues are removed. It is thus not surprising that the role longitudinal chromatic aberration plays in emmetropization is equally confusing. On this basis greater variability in the emmetropizing responses of chicks exposed to "normal vision" under monochromatic light may be expected. The variability of results was similar for all interrupted occlusion groups at day 5; however at day 10 slightly greater variability did occur for the blue light treatment group compared with bright white light group.

5.3.5. Conclusions.

In a rich stimulus environment adequate information is available under monochromatic conditions to guide emmetropization in the chick, whether the monochromatic light is from either extreme or from the centre of the visible spectrum. This result lends further support to the contention that there are alternative non-chromatic cues to defocus.

CHAPTER 6

THE ROLE OF CONTRAST AND SPATIAL FREQUENCY IN EMMETROPIZATION

6.0. Contrast and Spatial Frequency

There is increasing evidence for an active emmetropization process in chicks. While large refractive errors, usually high hyperopia, are often present in newly hatched chicks, these refractive errors rapidly decrease during development (Wallman *et al.*, 1981). Furthermore, chickens also show compensatory alterations in eye growth in response to artificially induced refractive errors, with myopic defocus resulting in hyperopia and hyperopic defocus resulting in myopia (Schaeffel *et al.*, 1988; Irving *et al.*, 1991).

Disruption of the natural emmetropization process occurs when young chicks are deprived of high quality form vision, resulting in excessive axial eye growth and high myopia (Wallman *et al.*, 1978b). This anomalous eye growth is prevented by intermittent normal "visual stimulation"; as little as 2 hrs of normal vision per day totally prevents form-deprivation myopia (Nickla *et al.*, 1989) and even 20 mins of normal "visual stimulation" per day results in a significant reduction (section 3.2). As a working hypothesis, it is presumed that the state of defocus of the eye is analyzed during the period of normal vision, information somehow being translated into an appropriate modulatory eye growth signal.

While the above observations would suggest the existence of a visually guided, active emmetropization process, the nature of the visual cues for defocus that guide this process remains obscure. Two possible cues to defocus that have been previously investigated are accommodation and chromatic aberration. These have been discussed in more detail in Chapters 4 and 5. However, the observations that lesions of the Edinger-Westphal nucleus (Schaeffel *et al.*, 1990) and section of the ciliary nerve (section 4.1) have little effect on the ability of chicks to compensate for lens-induced defocus would indicate that active accommodation is not essential to the emmetropization process. The fact that recovery from form-deprivation myopia occurs in monochromatic

light (Wildsoet *et al.*, 1993; section 5.2) would also suggest that chromatic aberration is not a fundamental cue for the emmetropization process.

Based on current evidence, it is reasonable to assume that emmetropization is a local ocular phenomenon and that the defocus cue *must be detected locally. Optic nerve section does not prevent the* recovery from form-deprivation myopia towards emmetropia (Wildsoet and Pettigrew, 1988; Troilo and Wallman, 1991) and in chicks raised with partial occluders, myopia is restricted to the visually deprived field; the non-deprived field remains near emmetropia (Wallman and Adams, 1987). These data suggest that the correct direction for eye growth, at least at a gross level, must be detected by the retina.

In this chapter the role that contrast (section 6.1) and spatial frequency (section 6.2) play in emmetropization was investigated using the same interrupted-occlusion paradigm used to investigate the role of longitudinal chromatic aberration (section 5.3). The main question for this study was: do contrast and spatial frequency provide information about defocus for the emmetropization mechanism? To address this question, the nature of the visual information presented during periods of normal vision in the interrupted-occlusion paradigm was manipulated; in particular, the effects of restricted contrast and restricted spatial frequency environments on form-deprivation myopia were investigated. It should be noted that while contrast or spatial frequency information was manipulated there was no attempt to control other aspects of the visual environment, e.g. lighting.

6.1. The Ability of Limited Contrast Environments to Reduce Occlusion-Induced Myopia in Chicks

6.1.0. Summary

In this study, the role of contrast as a visual cue for emmetropization was investigated. Chicks were either constantly occluded or the occlusion was interrupted with 20 mins of "visual stimulation" per day; chicks were exposed to either: i) a normal visual environment, or ii) a restricted contrast environment. Constant occlusion resulted in –12.0 D of myopia at day 5; this was reduced to –3.6 D when periods of normal vision were introduced. Visual stimulation in a high-contrast environment (87% contrast) decreased form-deprivation myopia to –4.8 D, a medium-

contrast environment (38% contrast) decreased it to –4.4 D, a low-contrast environment (4% contrast) to –6.9 D and a mixed-contrast environment (all three contrasts) to –5.2 D. Restricted contrast environments were as effective as the normal visual environment at reducing the magnitude of occlusion-induced myopia. The data indicate that a varied contrast environment is not a requirement for emmetropization.

6.1.1. Introduction

It has been suggested that the contrast of the visual image could be used as a guide to defocus; the contrast of a blurred, out-of-focus image is reduced in proportion to the magnitude of defocus (Campbell and Westheimer, 1965) and thus such changes in contrast could signal defocus. The rate of decrease of contrast sensitivity with defocus varies with spatial frequency. Optical defocus has little effect on contrast sensitivity for very large or very low spatial frequencies, i.e coarser than between 3 and 5 cycles/deg (Campbell and Westheimer, 1965). However, optical defocus produced by uncorrected myopia has a very large effect on the contrast sensitivity of high spatial frequencies. The contrast of spatial frequency gratings greater than 30 cycles/deg is subthreshold with less than 2 D of defocus, the equivalent value for 22 cycles/deg is 3 D. Reported decreases of contrast sensitivity measured using a Pelli-Robson chart were 0.09 log units per diopter (Bradley *et al.*, 1991).

The importance of contrast as a defocus cue is suggested by studies of the contrast dependence of the human accommodation system. Although conflicting results have been obtained, with some studies reporting that the accommodation response is not greatly influenced by contrast (Charman and Tucker, 1978b; Ciuffreda and Rumpf, 1985; Tucker *et al.*, 1986) and others showing that the accommodation response is less accurate with reduced contrast stimuli (Raymond *et al.*, 1984; Wolfe and Owens, 1981), it is now well accepted that there is a threshold contrast level below which the accommodative system is inaccurate. The accuracy of the accommodative response is thus initially relatively unaffected by a reduction in stimulus contrast from high values (Ward, 1987). Similarly, there may be a contrast threshold for the emmetropization system below which inadequate defocus cues are provided.

The finding that humans can accurately accommodate to a sinusoidal grating (Charman and Tucker, 1977b; Owens, 1980), where defocus attenuates contrast but does not affect the shape of the waveform,

has raised the possibility that accommodation operates as a contrast maximizing feedback loop (Kotulak and Schor, 1986a). Kotulak and Schor (1986a) suggested that an increase in retinal-image contrast with increasing accommodation would signal an increase in intraocular lens power was required to improve focus and a decrease in contrast with increasing accommodation signalled the reverse. Perhaps emmetropization could operate as a maximizing contrast feedback loop with low contrast signalling the presence of defocus.

The visual cue for emmetropization must be detectable by the retina; changes in contrast meet this criterion. Between the photoreceptors, which detect the visual image, and the ganglion cells, which transmit the visual signal to the brain, complex visual processing occurs (Dowling, 1987). The retina processes contrast information, which is degraded by optical defocus, and thus the eye has access to defocus information.

The role of contrast in the emmetropization process was investigated using restricted-contrast visual environments. As a natural visual environment consists of a rich array of contrasts, emmetropization may be contrast dependent, with a mixed array of contrasts providing the best defocus cues. Thus environments of limited contrast variability were selected for study. As low contrast stimuli below a threshold level may be inadequate for emmetropization a low contrast environment was also included. Chicks were form deprived and the ability of restricted-contrast environments to guide emmetropization, i.e. prevent form-deprivation myopia was studied.

6.1.2. Methods

Animals

Day-old male White Leghorn-New Hampshire cross chicks were monocularly occluded for 10 days from hatching. Chicks were either constantly occluded (CO) or the occlusion was interrupted with 20 mins of "visual stimulation" per day. During the period of "visual stimulation", chicks were exposed to either i) a normal visual environment (NV) or ii) a restricted contrast environment. All chicks were reared in temperature-controlled (30°C) cages under a 12 hr light (white light, 250 lux) / 12 hr dark circadian cycle and food and water were provided *ad libitum*.

In addition, a pilot study was undertaken to confirm that any myopic change produced by occlusion did not initially interfere with the visibility

of the stimuli; for this study, 6 chicks were occluded from day 1, their refractive error measured on day 2 and their behavioural responses to the visual stimuli studied.

Visual stimuli

In each case, the stimulus was generated using a computer. Hard copies were obtained and the stimulus attached to the walls of a white, cylindrical drum. Three different contrasts were used. These were presented alone or combined to give four different limited contrast environments, the characteristics of which are summarized in Table 6.1.1. The only difference in the stimuli was their contrast, all consisted of a random arrangement of shapes from as large as 0.086 cycles/deg and included sharp edges as a source of high frequency information. The three contrast levels used were: 87%, i.e. high-contrast (HC, virtually black on white), 38%, i.e. mid-contrast (MC), and 4%, i.e. low-contrast (LC). A fourth pattern was generated by mixing all three contrast levels (MXC). Thus contrast was manipulated, without removing high spatial frequency information, in an attempt isolate the role of image contrast as an emmetropizing cue.

Table 6.1.1. Characteristics of the restricted contrast visual environments.

Visual stimulus	Contrast (%)	Mean luminance (normalized)	Object size (cycles/deg)	Object orientation
High	87	0.75	0.086 and smaller	Random
Medium	38	0.55	0.086 and smaller	Random
Low	4	1	0.086 and smaller	Random
Mixed	4, 38, 87	mixed	0.086 and smaller	Random

Stimulus contrast as determined from measurements of the luminance of the darker and lighter components of the patterns using a Hagner photometer; these values were substituted into the Michaelson formula for contrast, i.e. $(L_{max} - L_{min})/(L_{max} + L_{min})$ where L_{max} and L_{min} were the maximum and minimum luminances of the stimuli, respectively. The mean luminance of the stimuli, $(L_{max} + L_{min})/2$, was also calculated; the

latter values for each contrast environment were subsequently normalised, such that the stimulus having the brightest mean luminance was given a value of 1.

In addition, the normal visual environment (NV) consisted of the chicks being placed in their usual caged enclosure (250 lux, other chicks, food, food containers) during occluder removal.

Presentation of visual stimuli

The required visual stimulus was attached to the inside of a white, acrylic, cylindrical drum (50 cm diameter, 50 cm height; Plate 6.1.1). A lid, made of the same material as the drum allowed diffuse light into the drum while excluding distracting stimuli. Chicks were physically restrained, using a neck brace attached to a small container; this prevented the chicks from running around within the drum but allowed lateral head movements of approximately 90 degrees. In addition, a collar of the same pattern as the higher luminance portion of the visual stimulus, was placed around the chick's neck to prevent it from viewing itself. The chicks was placed inside the drum and its occluder removed. The drum, but not the chick, was rotated at a speed of 1 revolution/min for 2 mins in a clockwise direction and then 2 mins in a counter clockwise direction for a total time of 20 mins. This strategy was adopted in an attempt to maintain chick interest and fixation on the stimulus, young chicks otherwise tended to sleep under such conditions. At the end of this period, the chick's occluder was replaced, the chick removed from the drum and the chick returned to its usual enclosure.

For the visibility study, testing in the drum occurred on days 1 and 2. The 6 chicks assigned to this pilot study were placed in turn into the drum. The speed of the drum was increased to 2.0 revs/min and the eyes and head of each chick observed to determine if optokinetic nystagmus (OKN) could be elicited in response to the stimulus. Optokinetic responses to rotating visual targets have been previously reported for chicks (Wallman and Velez, 1985; Bonaventure *et al.*, 1992). This procedure was repeated for all three contrast levels.

Plate 6.1

(overleaf)

Plate 6.1. Examples of the restricted contrast and restricted spatial frequency stimuli. In **A**. the mixed-contrast stimulus is shown lining the inside of the plastic drum in which chicks receive "visual stimulation" and in **B**. the high/low-spatial frequency stimulus is shown.

A
B

Measurements

On days 5 and 10, refractive errors (on axis) and axial ocular dimensions were measured under halothane anaesthesia using retinoscopy (non-cycloplegic) and A-scan ultrasonography respectively on all chicks. Using A-scan ultrasonography anterior chamber depth (ACD), axial lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. In addition, to determine the presence or otherwise of refractive asymmetry, the refractive errors of treated eyes and 10 randomly selected normal eyes were also measured off-axis at approximately 40 degrees into both the nasal and temporal visual fields using retinoscopy. These eccentricities represent the greatest compatible with a clear, usable retinoscopic reflex. Corneal power (CP) was measured by infrared-video-photokeratometry under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Chicks were finally sacrificed using sodium pentobarbitone. The eyes were enucleated, cleared of extraneous muscle tissue and the axial length and equatorial diameters measured directly with digital calipers. Eyes were also weighed on an electronic balance (see Appendix I for more details).

The 6 chicks assigned to the visibility study were refracted on day 2, at the time that the first period of visual stimulation would have been given, to estimate the refractive errors likely to be present at the commencement of the presentation of the restricted-contrast visual stimuli.

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between treated (T) and normal (N) eyes of the same animal, the Wilcoxon matched-pairs signed ranks-test was used (WSRT). The Mann-Whitney U-test was used (MWUT) to assess the difference between different contrast treatment groups and either the NV group or CO group; interocular differences were used for this analysis (see Appendix I for more details). Data are presented as mean ± SD unless otherwise stated.

6.1.3. Results

Visibility study

Refractions of chicks on day 2, at the time when the first period of visual stimulation would have been given, gave a mean refractive error of +2.0±1.0 D for occluded eyes and +3.6±0.9 D for normal eyes (Fig. 6.1.1). The range of refractions was +0.5 D to +3.3 D for treated eyes and +2.8 D to +5.0 D for normal eyes.

Optokinetic nystagmus, OKN, was observed in 5/6, 6/6, 6/6 chicks at day 1 prior to occlusion for the low-, mid- and high-contrast stimuli respectively. Performance improved to 6/6 for all treatment groups at day 2.

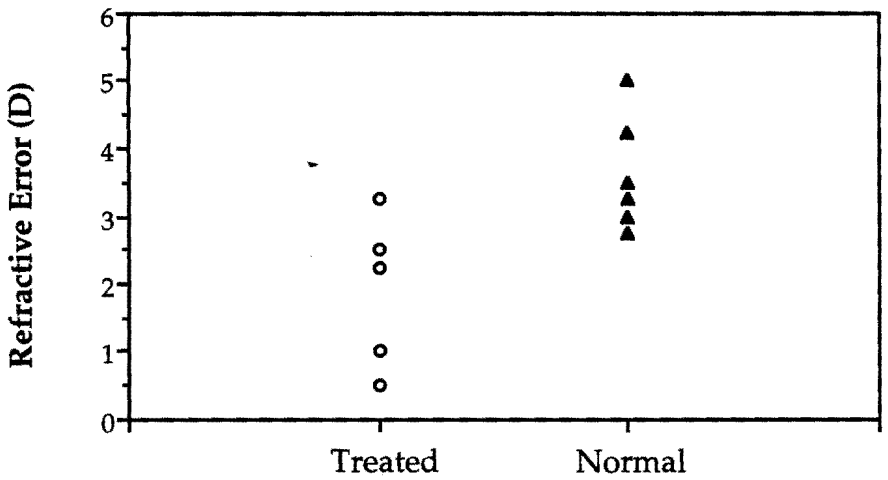


Figure 6.1.1. Refractive error of treated and normal eyes at day 2; some points overlap.

Constant occlusion

After 5 days of constant monocular form deprivation, both the anterior and vitreous chambers of treated eyes were significantly deeper, the cornea was significantly steeper and eyes were highly myopic (interocular differences, Table 6.1.2; $P < 0.005$, WSRT, for all cases except CP, $P < 0.05$, WSRT; see Appendix II, Tables AII.6.1 for treated and normal eye data). The average occlusion-induced form-deprivation refractive response was −12±3.0 D (Table 6.1.4). Anterior chamber deepening of 0.06±0.05 mm,

vitreous chamber elongation of 0.49±0.10 mm, and corneal steepening of 1.46±1.6 D contributed to the measured refractive error.

Maintaining the deprivation to 10 days resulted in even greater effects; there was an increased expansion of anterior and vitreous chambers, the cornea showed greater steepening and, as a consequence, eyes were more myopic (Table 6.2.2; $P < 0.005$, WSRT, for all cases except CP, $P < 0.05$, WSRT). At this time point, the mean refractive error of treated eyes was –19±6 D, mean ACD and VCD deepening was 0.13±0.15 mm and 0.82±0.20 mm respectively and corneal steepening was 3.5±2.4 D. There was no effect of occlusion on measured axial lens thickness at either time point.

Predicted changes in refractive error based on measured changes in ACDs and VCDs were very similar to those actually measured at both day 5 and 10 (Table 6.1.3). This analysis confirmed that vitreous chamber elongation contributed most to the myopic shift seen with constant deprivation.

Table 6.1.2. Differences in ocular parameters between treated and normal eyes after 5 and 10 days of constant form deprivation (mean ± SD, n = 7 both groups).

Ocular parameter	Day 5	Day 10
Δ Refraction (D)	–12.0±3.0***	–19±6***
Δ Corneal power (D)	+1.46±1.6*	+3.5±2.4*
Δ Anterior chamber depth (mm)	+0.06±0.05***	+0.13±0.15***
Δ Axial lens thickness (mm)	+0.006±0.01	+0.006±0.02
Δ Vitreous chamber depth (mm)	+0.49±0.10***	+0.82±0.20***
Δ Axial length (mm)	+0.55±0.12***	+0.96±0.22***

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test.*

Table 6.1.3. Predicted (based on ocular parameter changes) compared with measured changes in refractive error (RE) for constant occlusion (CO) at day 5 and 10.

	Day 5	Day 10
Measured Δ RE (D)	−12.0±3.0	−19±6
Δ RE ACD (D)	−1.7	−3.8
Δ RE VCD (D)	−7.8	−13.0
Measured Δ CP (D)	+1.46±1.6	+3.5±2.4
Predicted Δ RE (D)	−11.0	−20.3

Based on schematic eye data of Schaeffel and Howland, (1988a; see Appendix I for details).

Effect of daily periods of normal visual stimulation on form-deprivation myopia

Short daily periods of normal vision, under bright white light (NV; 250 lux), significantly reduced the magnitude of form-deprivation myopia compared with constant occlusion (CO; interocular differences, Table 6.1.4; $P < 0.005$, MWUT; see Appendix II, Tables AII.6.1, for treated and normal eye data). The extreme myopia, i.e. −12.0±3.0 D at day 5, seen with constant deprivation was decreased to 30%, i.e. −3.6±1.5 D, when occlusion was interrupted by periods of normal vision. The refractive error of treated eyes was −1.5±2.0 D compared with +2.2±1.4 D for normal eyes. Similar differences between the CO and NV group, i.e. to 30% were also observed at 10 days. Constant occlusion after this longer period resulted in a −19±6 D myopic shift, compared with −5.6±3.0 D when the occlusion was interrupted ($P < 0.005$, MWUT). In absolute terms, the residual myopia of treated eyes was also greater at 10 days (mean refractive error of −4.7±3.1 D compared with −1.5±2.0 D at day 5 for the NV group).

The decrease in the myopic shift when occlusion was interrupted with periods of normal visual stimulation, was due to the period of normal vision reducing the amount of exaggerated growth of the vitreous chamber (Fig. 6.1.3, day 5; Fig. 6.1.5, day 10) and hence AL (Fig. 6.1.2, day 5; Fig. 6.1.4, day 10) compared with that seen with CO. Short periods of normal visual stimulation reduced the vitreous chamber expansion to 40%, from 0.49±0.1 mm for CO to 0.20±0.03 mm ($P < 0.005$,

MWUT); axial expansion was similarly reduced from 0.55±0.12 mm for CO to 0.21±0.05 mm ($P < 0.005$, MWUT), at day 5. Similar reductions were seen at day 10, i.e. to 40% of CO levels, for both the VCD and AL.

There was a discrepancy between changes in VCD and AL dimensions, i.e. AL changes exceeded vitreous chamber changes. This discrepancy reflected the effects of occlusion on the anterior chamber. Constant occlusion caused deepening of the anterior chamber and a similar effect, though reduced, was also seen when the occlusion was interrupted by normal vision (Fig. 6.1.3, day 5; Fig. 6.1.5, day 10). The deepening of the anterior chamber was decreased, from 0.06±0.05 mm for CO to 0.03±0.05 mm for the NV treatment group, i.e. by 50% of CO level ($P < 0.01$, MWUT), at day 5. Comparable figures at day 10 were 0.13±.15 mm and 0.08±0.04 mm, ($P < 0.05$, MWUT), i.e. a reduction to 60% of CO levels. ALT was unaffected by occlusion and there was also no effect of interrupted occlusion on the lens (Fig. 6.1.3, day 5; Fig. 6.1.5, day 10). The corneal steepening seen with occlusion was not observed with interrupted occlusion at either day 5 or 10 (Fig. 6.1.6)

Table 6.1.4. Predicted (based on ocular parameter changes) compared with measured changes in refractive error (RE) when occlusion is interrupted by periods of normal vision (NV), at day 5 and 10.

Ocular parameter	Day 5	Day 10
Measured Δ RE (D)	–3.6±1.5 D	–5.6±3.0 D
Δ RE ACD (D)	–0.8	–2.3
Δ RE VCD (D)	–3.2	–5.7
Measured Δ CP (D)	–0.9±2.6	0±1.4
Predicted Δ RE (D)	–3.1	–8

Based on schematic eye data of Schaeffel and Howland (1988a, see Appendix I for details).

For interrupted occlusion predicted changes in refractive error based on measured changes in ACD, VCD and corneal power at day 5 were very similar to those measured using retinoscopy (Table 6.1.4). However at day 10, the predicted changes in refraction based on anterior and vitreous chamber expansion were slightly higher than those actually measured.

Vitreous chamber elongation contributed most to the myopic shifts observed.

Effect of short daily periods of visual stimulation with restricted contrast environments on form-deprivation myopia

When, instead of a normal visual environment, chicks were exposed to a restricted contrast environment during the 20 min period of occluder removal, the effects of form deprivation were reduced (see Appendix II, Tables AII.6.1, for treated and normal eye data). With the exception of the LC group, restricted contrast environments generally proved to be as effective as normal vision in preventing form-deprivation myopia.

Short periods of exposure to the HC stimuli (87% contrast) decreased myopic shifts due to occlusion to –4.8±1.2 D, i.e. 40% of CO level ($P<0.005$, MWUT), and to –6.7±2.1 D, i.e. 35% of CO level ($P < 0.005$, MWUT), after 5 and 10 days of treatment respectively. Similar reductions were recorded for exposure to the MC stimulus (38% contrast), i.e. to 35% and 30% of CO level at days 5 and 10 respectively. These are equivalent to myopic shifts of –4.4±1.5 D ($P < 0.005$, MWUT) and –6.0±3.0 D ($P < 0.01$, MWUT). The picture was slightly different for the LC condition, the LC stimulus (4% contrast) was significantly worse than normal vision at preventing form-deprivation myopia. Myopic shifts of –6.9±1.2 D, i.e. 60% of CO level ($P < 0.005$, MWUT) and –8.2±5.0 D, i.e. 45% of CO level ($P < 0.005$, MWUT) being recorded at days 5 and 10 respectively. However, although the LC stimulus was worse than NV, it was not significantly different from any of the other restricted contrast groups. Exposure to all three contrasts, i.e. to the MXC stimulus, resulted in myopic shifts of –5.2±1.2 D, i.e. 40% of CO level ($P < 0.005$, MWUT) and –7.8±3.0 D, i.e. 40% of CO level ($P < 0.01$, MWUT), at day 5 and 10 respectively (Table 6.1.5; Fig. 6.1.2, day 5; Fig. 6.1.4, day 10).

As for normal visual stimulation, reductions in occlusion-induced myopia by short periods of visual stimulation with the restricted-contrast environments could be attributed to changes in vitreous chamber and hence AL responses to occlusion. Deprivation-induced vitreous chamber elongation was decreased from 0.49±0.2 mm (CO) to 0.29±0.14 mm (60% of CO level), 0.32±0.12 mm (65% of CO level), 0.32±0.14 mm (65% of CO level) and 0.31±0.10 mm (65% of CO level) with short daily visual stimulation with HC, MC, LC and MXC stimuli respectively, at day 5 (Fig 6.2.3). While all the stimuli reduced vitreous elongation, they were less

effective than normal vision ($P < 0.05$, MWUT). Similar decreases in percentage terms were measured at day 10, i.e. from 0.82±0.20 mm (CO) to 0.48±0.12 mm (60% of CO level), 0.41±0.14 mm (50% of CO level), 0.52±0.20 mm (65% of CO level) and 0.47±0.20 (60% of CO level) respectively (Fig. 6.1.5). However, at this later time point the values pertaining to the restricted contrast conditions were not significantly different from those obtained with NV. Also consistent with the trend at day 5, there was no difference in the magnitude of vitreous elongation between the different contrast treatment groups at day 10.

The deepening of the anterior chamber seen with constant occlusion was reduced by the introduction of short periods of visual stimulation. Increased anterior chamber growth was reduced at day 5, from 0.06±0.05 mm (CO) to 0.03±0.03 mm (50% of CO level), 0.01±0.03 mm (15% of CO level), 0.03±0.04 mm (50% of CO level) and 0.03±0.03 mm (50% of CO level) for HC, MC, LC and MXC stimuli respectively (Fig. 6.1.3). Comparable figures at day 10 were 0.13±0.15 mm, 0.05±0.05 mm (40% of CO level), 0.05±0.10 mm (40% of CO level), 0.04±0.07 mm (30% of CO level) and 0.06±0.04 mm (45% of CO level) for CO, HC, MC, LC and MXC treatment groups respectively (Fig. 6.1.5). There was no significant difference in the degree of residual anterior chamber deepening for the different contrast treatment groups at either age.

ALT was unaffected by form deprivation, and there was also no effect of introducing periods of restricted-contrast visual stimulation on lens thickness (Fig. 6.1.3, day 5; Fig. 6.1.5, day 10). Similar to NV, occlusion-induced corneal steepening was not seen at either age when occlusion was interrupted with the restricted-contrast environments. Slight corneal flattening was observed; however this was not significantly different for the different contrast treatment groups.

Table 6.1.5. Effect of short periods of visual stimulation on occlusion-induced form-deprivation myopia. Differences in refraction of treated and normal eyes measured at days 5 and day 10 (mean ± SD, n).

	Δ Refraction (D)	
Treatment group	**Day 5**	**Day 10**
Constant occlusion (CO)	–12.0±3.0, 7	–19±6, 7
Normal vision (NV)	–3.6±1.5, 7***	–5.6±3.0, 6***
High-contrast (HC)	–4.8±1.2, 10***	–6.7±2.1, 9***
Medium-contrast (MC)	–4.4±1.5, 10***	–6.0±3.0, 7***
Low-contrast (LC)	–6.9±1.2, 10***•	–8.2±5.0, 8***•
Mixed-contrast (MXC)	–5.2±1.2, 10***	–7.8±3.0, 7***

*Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted contrast groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).*

Table 6.1.6. Predicted (based on ocular parameter changes) compared with measured changes in refractive error (RE) for occlusion interrupted by restricted contrast visual stimulation, at days 5 and 10.

	Day 5				Day 10			
	HC	**MC**	**LC**	**MXC**	**HC**	**MC**	**LC**	**MXC**
Measured Δ RE (D)	–4.8±1.2	–4.4±1.5	–6.9±1.2	–5.2±1.2	–6.7±2.1	–6.0±3.0	–8.2±5.0	–7.8±3.0
Δ RE ACD (D)	–0.9	–0.3	–0.9	–0.9	–1.5	–1.5	–1.2	–1.7
Δ RE VCD (D)	–4.6	–5.1	–5.1	–4.9	–7.6	–6.5	–8.2	–7.4
Measured Δ CP (D)	–1.0±3.6	–0.4±3.3	–0.1±1.8	+0.1±2.1	–1.2±1.8	–1.1±3.9	–1.2±1.8	+0.5±3.0
Predicted Δ RE (D)	–4.5	–5.0	–5.9	–5.9	–7.9	–6.9	–8.2	–9.6

Based on schematic eye data of Schaeffel and Howland (1988a, see Appendix I for details).

Predicted changes in refractive error based on measured changes in ACD and VCD and corneal power were generally similar to those measured using retinoscopy, for occlusion interrupted with restricted-contrast

stimulation at days 5 and 10 (Table 6.1.6). However for the restricted-contrast groups where the LC groups showed the greatest myopic shift there was no equivalent difference in the predicted magnitudes of myopic shifts for the different contrast treatment groups. This implies that perhaps some other factor, e.g. lens curvature, contributed to the greater myopia seen for the LC group. Vitreous chamber elongation contributed most to the myopic shift seen for all groups.

Effect on ocular symmetry

Refractive errors of treated eyes and 10 randomly selected normal eyes were measured using retinoscopy, both on-axis and approximately 40 degrees into the nasal and temporal fields. Refractions measured in the nasal visual field were significantly more myopic, i.e. by 0.6 D to 0.9 D, than those measured in the temporal field for all treatment groups (Table 6.1.7; $P < 0.005$, WSRT in all cases). The magnitude of nasal to temporal difference in refraction did not significantly differ between groups.

Table 6.1.7. Refractive errors measured across the retina; on axis, 40 degrees into the nasal visual field and 40 degrees into the temporal visual field, at day 5 (mean ± SD, n).

	Refraction (D)		
Treatment group	**On axis**	**Nasal field**	**Temporal field**
Constant occlusion	–9.5±2.8, 7	–9.8±2.9, 7*	–9.0±2.8, 7
Normal eyes	+2.6±1.3, 10	+2.3±1.3, 10*	+2.9±1.2, 10
Normal vision	–1.4±2.0, 7	–1.8±2.2, 7*	–1.1±2.0, 7
High-contrast	–2.3±2.2, 10	–2.7±2.2, 10*	–2.3±2.2, 10
Medium-contrast	–2.3±2.5, 10	–2.9±2.5, 10*	–2.3±2.4, 10
Low-contrast	–3.8±2.6, 10	–4.0±2.6, 10*	–3.3±2.6, 10
Mixed-contrast	–2.2±1.8, 10	–2.3±1.9, 10**	–1.4±2.2, 10

*Differences between nasal and temporal visual field refractions significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test. Differences between nasal and temporal visual field refractions were not significantly different for the different treatment groups Mann-Whitney U-test.*

Enucleated eye data

The trends in the ultrasound axial length obtained at day 10, were confirmed by external measurements of axial length (Fig. 6.1.7). The mean external axial length of constantly occluded eyes was 9.95±0.38 mm compared with 9.11±0.26 mm for contralateral normal eyes; this represents an average 0.84±0.48 mm increase in external axial eye growth with constant occlusion. Although external axial length measurements were more variable, they were not significantly different from those measured by ultrasound. The mean internal AL difference between treated and normal eyes was also very similar, i.e. 0.82±0.20 mm. When occlusion was interrupted, the magnitude of the axial increase was greatly reduced by at least 50%, regardless of whether occlusion was interrupted by stimulation in a normal visual environment or a restricted contrast environment ($P < 0.005$, MWUT all groups cf CO). There was no significant difference in residual axial elongation for the interrupted-occlusion-treatment groups.

In addition to causing axial expansion, constant occlusion also caused equatorial eye expansion, although the increases were not as great. The mean equatorial diameter of constantly occluded eyes was 12.27±0.37 mm compared with 11.94±0.30 mm for contralateral normal eyes; constant occlusion resulted in an average 0.33±0.28 mm increase in equatorial eye growth, only 40% of the magnitude of the recorded axial change. In contrast to the axial changes, equatorial expansion was not consistently decreased by interrupted occlusion and while only the HC treatment group showed a significant decrease (to 40% of CO value) in this effect ($P < 0.05$, MWUT), there was no statistically significant difference between interrupted-occlusion-treatment groups in this respect.

Constant occlusion also resulted in heavier eyes, i.e. increased wet eye weight. The wet eye weight of constantly occluded chicks was 0.72±0.06 g compared with 0.62±0.05 g for contralateral normal eyes, representing an average 0.1±0.06 g increase in weight. Like the axial changes, this effect was significantly decreased in all interrupted-occlusion-treatment groups ($P < 0.05$ all groups, except HC $P < 0.01$).

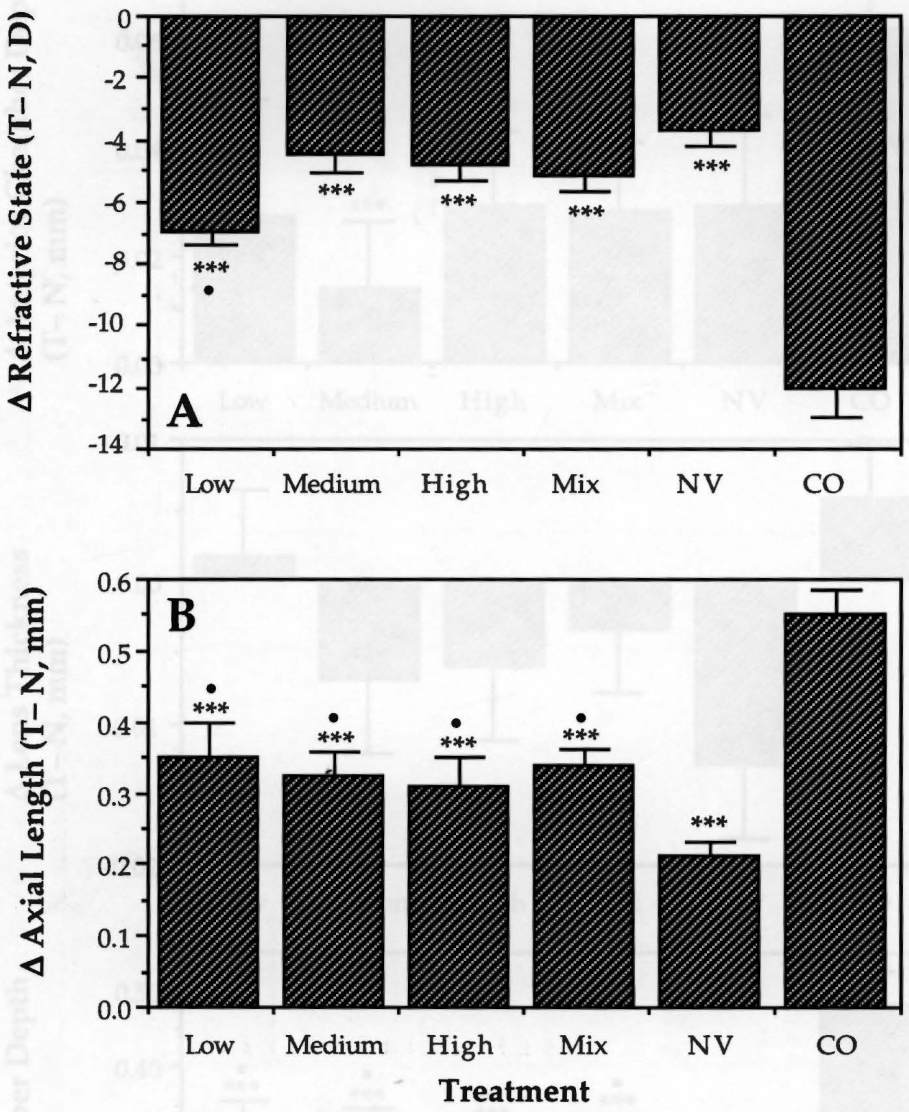


Figure 6.1.2. Differences (mean ± SE), at day 5, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted contrast groups significant at •P < 0.05, ••P < 0.01, •••P<0.005, Mann-Whitney U-test (one-tailed).

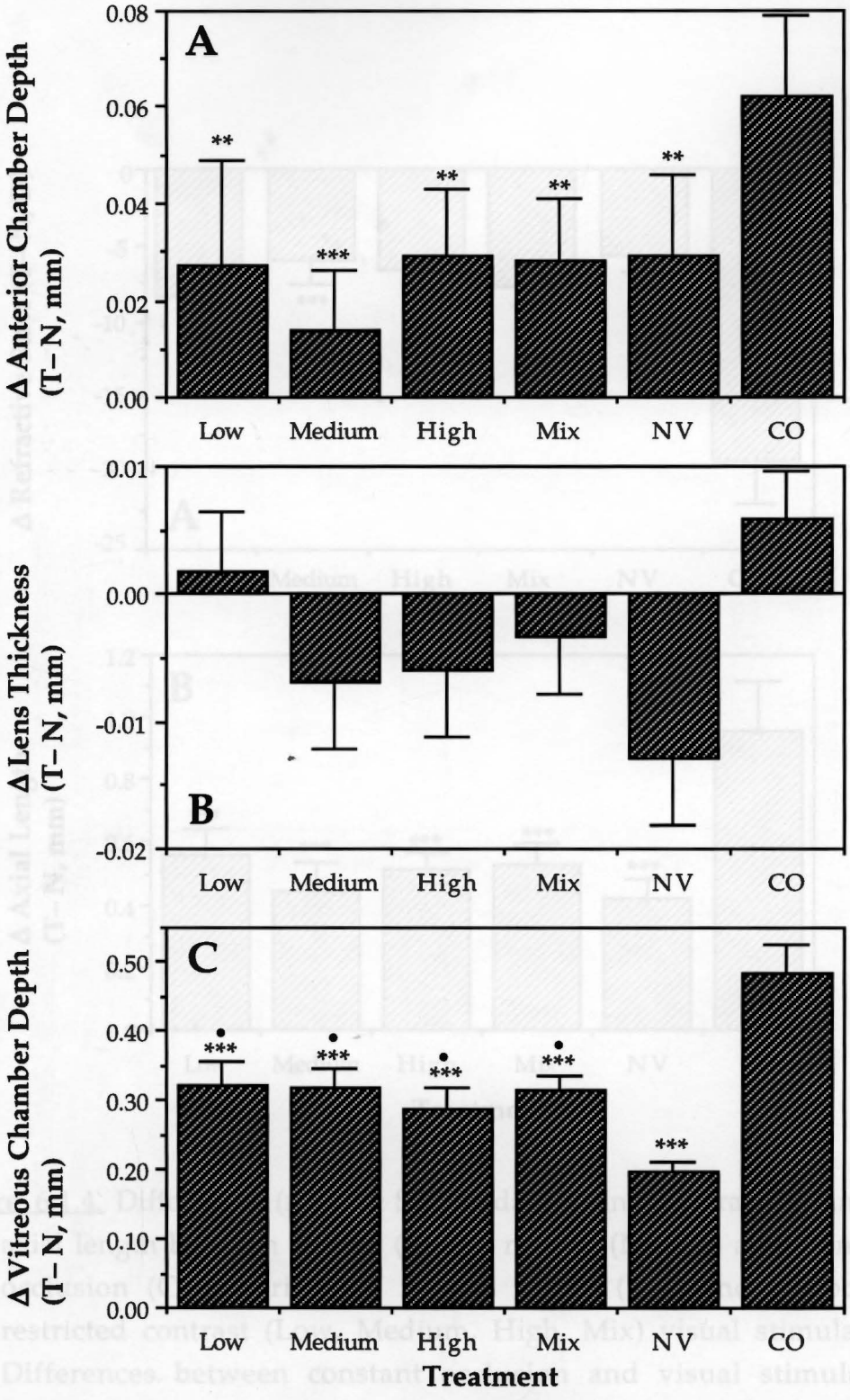


Figure 6.1.3. Differences (mean ± SE), at day 5, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted contrast groups significant at •P < 0.05, ••P < 0.01, •••P<0.005, Mann-Whitney U-test (one-tailed).

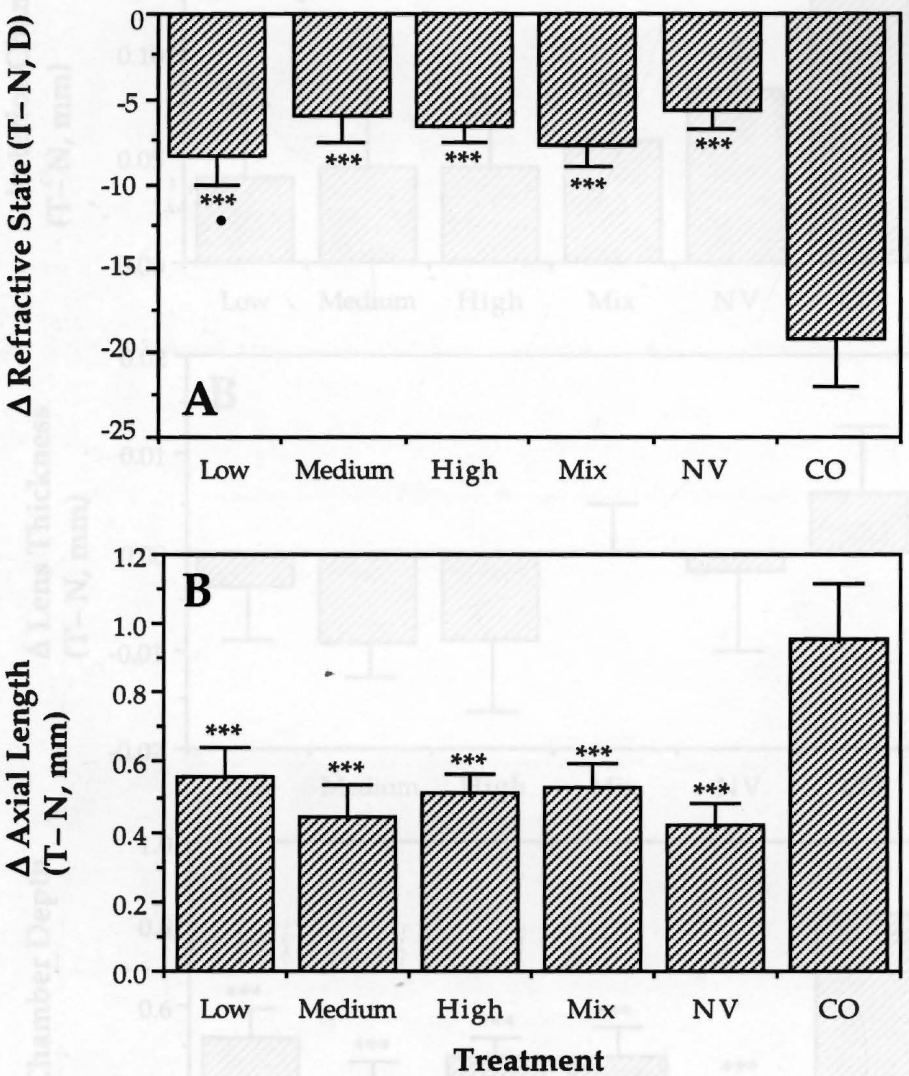


Figure 6.1.4. Differences (mean ± SE), at day 10, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted contrast groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

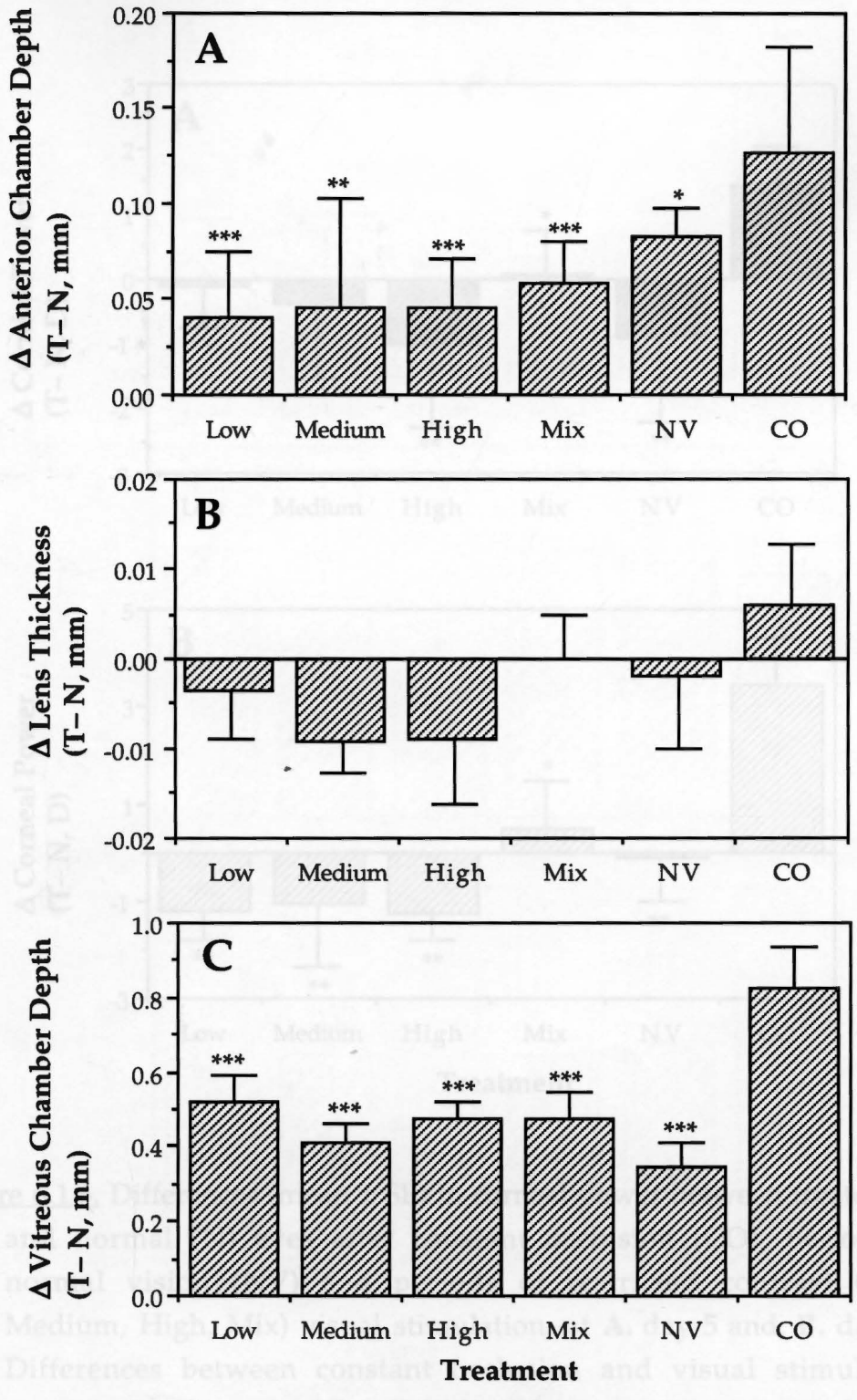


Figure 6.1.5. Differences (mean ± SE), at day 10, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

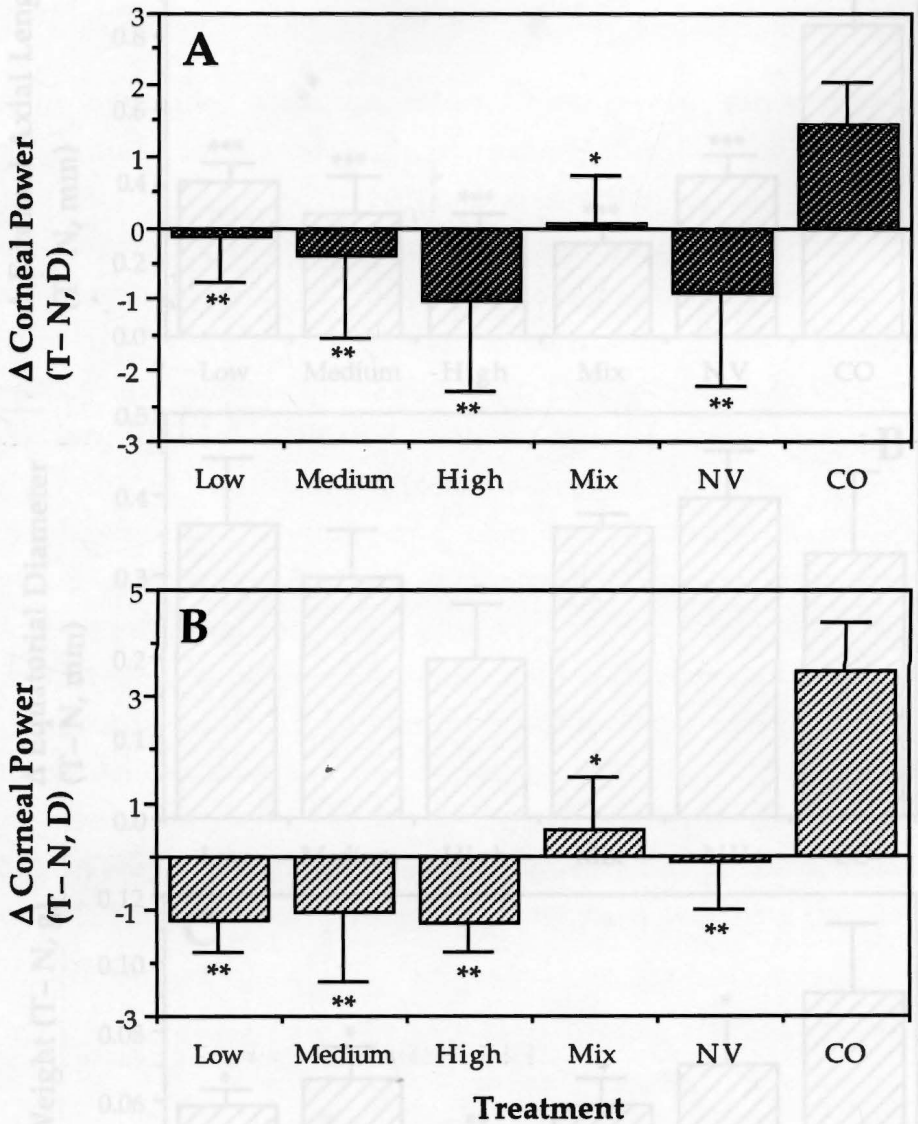


Figure 6.1.6. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation, at **A.** day 5 and, **B.** day 10. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

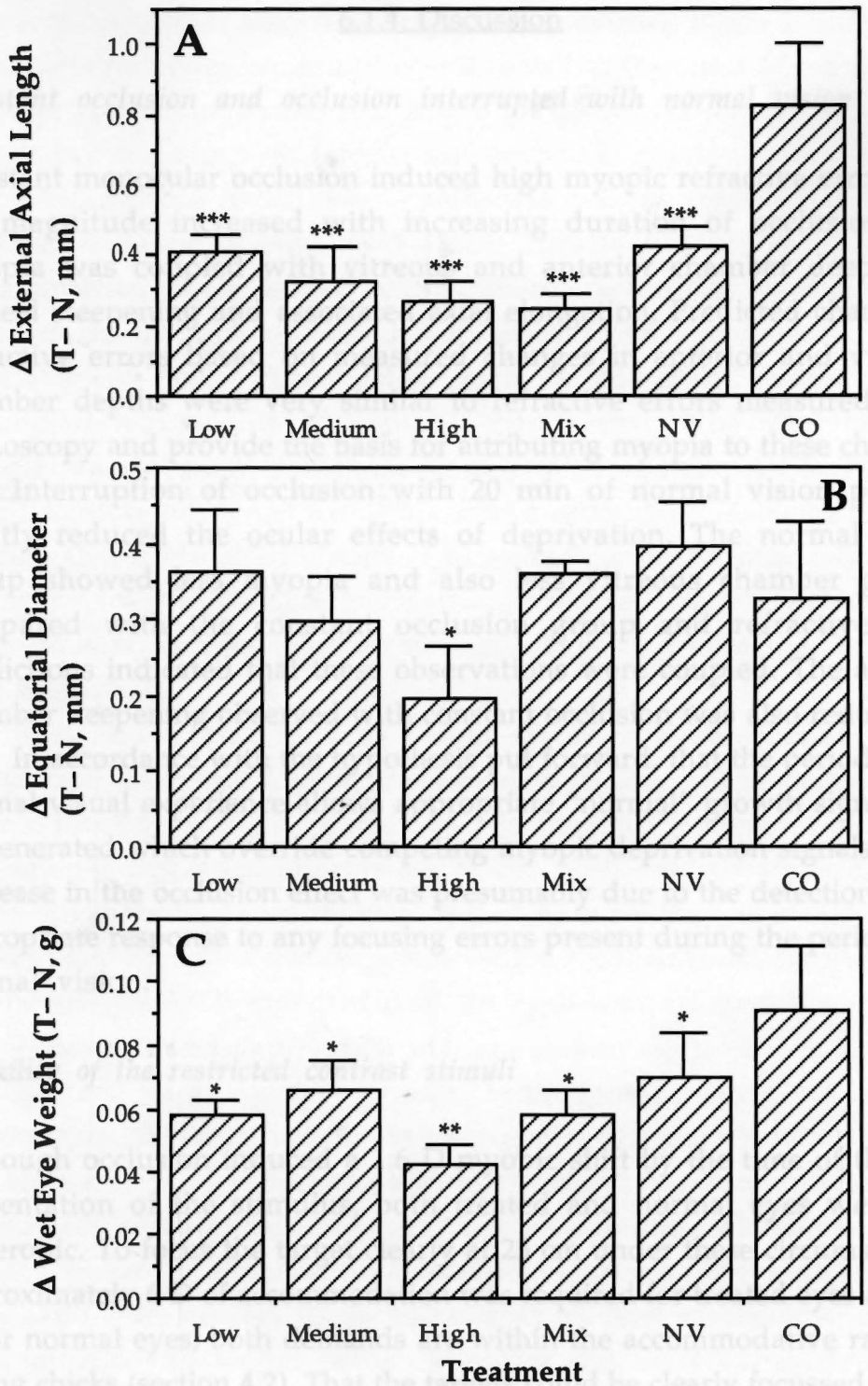


Figure 6.1.7. Differences (mean ± SE), at day 10, in **A.** external axial length, **B.** equatorial diameter and **C.** wet eye weight between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted contrast (Low, Medium, High, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, Mann-Whitney U-test (one-tailed).

6.1.4. Discussion

Constant occlusion and occlusion interrupted with normal vision

Constant monocular occlusion induced high myopic refractive errors and the magnitude increased with increasing duration of occlusion. The myopia was coupled with vitreous and anterior chamber deepening, corneal steepening and associated axial elongation. Predicted changes in refractive errors based on measured changes in anterior and vitreous chamber depths were very similar to refractive errors measured using retinoscopy and provide the basis for attributing myopia to these changes.

Interruption of occlusion with 20 min of normal vision per day greatly reduced the ocular effects of deprivation. The normal vision group showed less myopia and also less vitreous chamber growth compared with the constant occlusion group and refractive error predictions indicated that these observations were coupled. The anterior chamber deepening observed with constant occlusion was also reduced.

In accordance with the hypothesis put forward, that the period of normal visual experience allows appropriate "normal" growth signals to be generated which override competing myopic deprivation signals, the decrease in the occlusion effect was presumably due to the detection and appropriate response to any focusing errors present during the period of normal vision.

Visibility of the restricted contrast stimuli

Although occlusion induced a 1.6 D myopic shift by the time of the first presentation of the stimulus, both treated and normal eyes were still hyperopic. To focus the target clearly at 25 cm under these circumstances, approximately 6 D of accommodation was required for treated eyes and 7.5 D for normal eyes; both demands are within the accommodative range of young chicks (section 4.2). That the targets could be clearly focussed was of most importance for the low contrast target, where further contrast degradation due to blur may have rendered it "subthreshold". Observed optokinetic nystagmus responses to all targets further confirmed the prediction that the chicks were able to resolve all stimuli.

The role of contrast in emmetropization

This experiment was designed on the premise that a rich array of

contrasts, as encountered in a natural environment, might be important to the detection of defocus. In this case, blur has the effect of reducing the range of contrast and also reducing high contrast information. For the human eye the effect of defocus on contrast is quickly saturated, with equivalent contrast reductions for refractive defocus greater than about 4 D (Campbell and Westheimer, 1965). This would imply that while determinations of the magnitude of defocus above this level cannot be made on the basis of contrast change, the defocus signal should remain detectable. As one of these factors might be important, the experiment included environments of limited contrast variability, i.e. the high, medium and low contrast stimuli, and an environment that lacked high contrast information, i.e. the low contrast stimulus. However, the data indicate that a visual environment of varied contrast information is not required for the emmetropization process. Restricted contrast environments were as good as normal vision at preventing occlusion-induced myopia.

It is known that defocus would result in altered retinal image contrast, with defocus being associated with low image contrast and correct focus with high contrast. In addition to the contrast reduction high-spatial frequency information is lost with defocus and this effect may be of more relevance than the loss of contrast. If signs of defocus are ignored, this hypothesis would predict a greater than usual defocus signal for the low contrast compared with the high contrast stimulus and thus that although form-deprivation myopia should decrease for both low contrast and high contrast groups, reductions for the low contrast condition should be greater due to the presence of a greater defocus signal. The results did not support this with less effective emmetropization for the low contrast compared to high contrast stimulus. Alternatively, due to the choice of experimental conditions loss of the high contrast information the correct focus signal may be lost in the low contrast environment. This would predict a poorer and more variable response for the low contrast group; at 10 days refractive errors were on average 1.5 D more myopic for the low contrast group and much more variable.

Constant low image contrast may signal high hyperopia, as both distant and near targets outside of the range of accommodation would be blurred for the highly hyperopic eye. On the other hand, periods of both high and low image contrast may signal high myopia, as in this case image quality will be improved at near. This theory would predict that the low contrast environment would be less effective in preventing myopia due to a conflicting hyperopic defocus signal; trends in this

direction did occur. This theory can also be used to explain the high myopia seen with form-deprivation: both lid-suture and occluders produce constant low image contrast, an aberrant high hyperopic signal is generated and thus increased growth and myopia result.

An alternative explanation for the results obtained involves longitudinal chromatic aberration. The role that this plays in determining defocus remains unresolved and while in the previous section it was shown that emmetropization was not dependent on chromatic aberration alone as a cue to defocus, these studies do not rule out the possibility that chromatic aberration is important when alternative cues are eliminated. A recent study of Stone *et al.* (1993) which lends support to chromatic aberration being used by the human visual system in determining reflexive accommodation, might be of significance here. As mentioned in earlier sections (5.1, 5.2, 5.3), chromatic aberration causes a refractive difference between wavelengths, this may also mean that retinal image contrast is different for different wavelength components. If the longer wavelength component (i.e. red), is focussed, on the retina, then it will have the highest retinal image contrast with contrast decreasing for shorter wavelengths. If blue is focussed on the retina then it will have the highest contrast. Such differences in contrast between different wavelengths thus inherently specifies the magnitude and sign of defocus. As the different contrast stimuli were presented under white light, this means that, due to chromatic aberration, retinal images of varied contrast would result. That restricting the contrast of the stimuli does not prevent emmetropization can thus be explained if the chick eye can use its 3.7 D (section 5.1) of chromatic aberration to provide a defocus cue.

Emmetropization appears to involve local mechanisms. Thus the defocus cue for emmetropization must be detectable by the retina; changes in contrast meet this criteria. Complex visual processing occurs between the photoreceptors, which detect the visual image, and the ganglion cells, which transmit the visual signal to the brain (Dowling, 1987). The retina processes contrast information and at least in the monkey retina there appear to be two broad types of ganglion cells, one with high luminance-contrast sensitivity and the other with low luminance-contrast sensitivity (Kaplan and Shapley, 1986). In mammalian retina, colour opponent cells which receive differential input from the three cone types in the centre and surround regions of their receptive fields (Wiesel and Hubel, 1966; Dreher *et al.*, 1976; Schiller and Malpeli, 1977; DeMonasterio, 1978; Derrington, Krauskopf and Lennie, 1984) are sensitive to contrast (Shapley *et al.*, 1981; Schiller and Colby, 1983). If the proposed model for

emmetropization implicating longitudinal chromatic aberration is correct, it suggests that the retinal system must also be able to compare contrast experienced by different colour opponent channels. Although the presence of colour opponent channels has not been investigated in the chick, chick retina does contain five cone types: a UV photoreceptor, P506, P533, P569 and P606 (Bowmaker and Knowles, 1977), which theoretically could input into a defocus system based on colour perhaps involving colour opponent cells as described for monkey retina.

The visual manipulations used in the current studies have all involved very young chicks and thus the data indicate that the visual cue for emmetropization must be detectable by the visual system at an early age. While it is recognised that longitudinal chromatic aberration is a refractive phenomenon, if the input from different colour channels is to be used to analyse longitudinal chromatic aberration, functional colour vision must be present, at least to some degree. Although data on the emergence of colour vision is not available for the chick, colour vision seems to develop early in humans, with behavioural studies reporting colour discrimination in infants between 1 and 3 months of age (reviewed in Brown 1990; Morrone *et al.*, 1993). Linked to this it has also been recently reported that human infants have functional medium-wavelength-sensitive and long-wavelength-sensitive cones and the required post-receptor chromatic mechanisms to compare their signals (Allen *et al.*, 1993). This would suggest that if longitudinal chromatic aberration is used to guide emmetropization this would be possible from a young age.

Studies which show that emmetropization occurs in chicks reared under monochromatic light (Wildsoet *et al.*, 1993; Rohrer *et al.*, 1992; section 5.2 and 5.3), do not rule out the above LCA contrast model, chromatic aberration being only one of many potential defocus cues and the only one consistently controlled in these studies. However, it is also possible that some other process, not related to the above model, was occurring to prevent the development of form-deprivation myopia.

Significance for myopia due to visual deprivation

Form-deprivation treatments eliminate high spatial frequency visual information and considerably decrease the contrast of the retinal image. It has been postulated that excessive eye growth and myopia occur due to the contrast degradation and thus the open-looping of the visual feedback system produced by either lid suture or occlusion. These conditions differ from the low-contrast environment used in this study which was designed

to reduce contrast considerably without removing high spatial frequency information. Thus high spatial frequency cues were still provided but eyes were deprived of high-contrast cues. Two interpretations can be made of the low-contrast data: either it is the loss of high frequency information and not high contrast that is important in form-deprivation myopia or the low contrast condition was not of sufficiently low contrast to adequately simulate form-deprivation treatments. Contrast levels of less than 4% with occlusion or lid suture would be consistent with the latter interpretation. Although Hodos and Kuenzel (1984) demonstrated visually that occluders reduce the contrast of a square wave grating, the extent of contrast reduction was not reported. The relevance of such data is also questionable given that during the course of such experiments condensation and dust which invariably accumulate on the surfaces of the occluders further reduces contrast by variable amounts.

Significance of ocular symmetry study

It has been suggested that each part of the retina of the chick can control its refractive status and the growth of the underlying sclera (Wallman *et al.*, 1987). Hence based on the retinal ganglion cell topography of the chick (Ehrlich, 1981), it was predicted that the high contrast and mid contrast stimuli would be appropriate stimuli for the entire retina, but that the low contrast stimulus might be a poor stimulus for the retinal periphery, because assuming that such contrast levels are close to threshold, this stimulus would be the most affected by blur caused by any peripheral ocular aberrations. Following this line of logic, it is possible that the low contrast stimulus appears as an empty field to the peripheral retina which should therefore respond as if form deprived. To examine this possibility, off-axis as well as on-axis refractive errors were measured. Selective deprivation of the peripheral retina would have made off-axis refractions more myopic than on-axis refractions. However the data did not support this theory; refractions were always slightly more myopic when measured in the nasal field and this effect was not treatment specific. While it is assumed that 4% contrast may be close to threshold for the chick, the central contrast threshold for humans is substantially lower, i.e. 0.2% (reviewed in Woodhouse and Barlow, 1982). If the threshold for chicks is similarly low this could explain the lack of increased peripheral myopia. However, in the pigeon the peak contrast sensitivity is about 7% (reviewed in Hodos, 1993) which is substantially greater than that of the low contrast stimulus used in this study. It has been

suggested that the visual systems of birds are poor at detecting low contrast stimuli (reviewed in Hodos, 1993).

Interestingly, Millodot (1981) found in humans that the peripheral refraction was dependent on the central refractive error; emmetropes exhibited mixed astigmatism in the periphery, myopes compound myopic astigmatism and hyperopes compound hyperopic astigmatism (Millodot, 1981). These findings contrast with the result in chicks which suggests an anatomically-dependent nasal to temporal asymmetry. This may reflect different functional eye designs which support "good vision" in frontal and lateral fields in the case of the chick and only in the central field in humans.

"Transient" theory for myopia reduction

A theory to explain the decrease in myopia seen when occlusion is interrupted with the restricted contrast stimuli involves an analysis of retinal transients. Under normal conditions, images are sharply focussed and highly detailed, and normal eye movements cause such images to move across the retina. This image shift and associated bursts of retinal activity have been termed "transient" responses; normally there are many "transients". Occluders by decreasing contrast and the range of spatial frequencies contained within the image, also decrease the "transients", i.e. the information received before and after an eye movement would be near identical. It has been suggested that the reason why stroboscopic light rearing decreases the magnitude of form-deprivation myopia is that it artificially increases the number of "transients"(Gottlieb and Wallman, 1987). A potential pathway which could mediate such effects involves "Y/M" type cells which are sensitive to movement (reviewed in Perry *et al.*, 1990); these cells are thus also likely to detect transients (Gottlieb and Wallman, 1987). As the stimuli used in the current study were always moving, they too may have preferentially activated the equivalent of "Y/M" type cells in the chick and thereby decreased the form-deprivation response as observed. However, as chicks tracked the stimuli for greater than half the time, during which the retinal image would have been stable, this "strobe" effect is likely to contribute only in a minor way to the results reported here.

6.1.5. Conclusion

In conclusion, restricted contrast environments are as effective as normal vision in reducing the magnitude of occlusion-induced myopia. The data indicate that a varied contrast environment is not a prerequisite for emmetropization.

6.2. The Ability of Limited Spatial Frequency Environments to Reduce Occlusion-Induced Myopia in Chicks

6.2.0. Summary

In this study, the role of spatial frequency in emmetropization was investigated. Chicks were constantly occluded or the occlusion was interrupted with 20 mins of "visual stimulation" per day; chicks were exposed to either, i) a normal visual environment or ii) a restricted-spatial-frequency environment during this period of "visual stimulation". Constant occlusion resulted in –11.6 D (mean refractive error) of myopia by day 5 and this was reduced to –3.3 D when periods of normal vision were introduced. The effectiveness of restricted-spatial-frequency environments at reducing occlusion-induced myopia varied with the spatial frequency presented. The mid-spatial and mixed-spatial-frequency environments were as good as the normal visual environment at decreasing the effects of occlusion, with mean refractive errors of –4.0 D and –4.1 D being observed respectively. There was no significant difference in the magnitude of occlusion-induced myopia when occlusion was interrupted by high-spatial-frequency stimulation compared with that resulting from constant occlusion alone, i.e. –10.8 D compared with –11.6 D (mean refraction). The low-spatial-frequency stimulus was also poor at preventing occlusion-induced myopia with mean refraction being –7.3 D. Also, mixing the high- and the low-spatial-frequency stimulus was no more effective in reducing occlusion-induced myopia (mean refraction, –7.4 D) than those frequencies alone. The data indicate that emmetropization is dependent on spatial frequency, with mid-spatial-frequencies seemingly the most important for this process.

6.2.1. Introduction

Which spatial frequencies are important for eye growth? The conventional view is that the eye changes focus or alters growth so that fine-detailed images remain clear on the retina; detection of larger details is less dependent on accurate focus. It would seem logical that any emmetropization system would be tuned to respond to changes in the quality or amount of high-frequency information.

The spatial-frequency dependence of the eye-growth control system is unknown. However some information is known about the frequency tuning of the accommodation system from which analogies may be drawn, although similar attempts at examining the relationship between spatial frequency and accommodation in humans have produced conflicting results (Owens, 1980; Ciuffreda and Hokoda, 1983; Raymond *et al.*, 1984). Two different regions of the spatial-frequency spectrum have been suggested as the most important to the accommodative system. Early studies support the view that high spatial frequencies are required for accurate accommodative responses and are the basis of the "fine-focus" theory which suggests that the high spatial frequencies are required to "fine-tune" the accommodative response (Charman and Tucker, 1977b). However, results from later studies indicate that intermediate frequencies between 3 and 5 cycles/deg, i.e. around the peak of the contrast sensitivity function, are important (Owens, 1980; Ward, 1987). While this conflict remains unresolved these data do suggest that spatial-frequency information is in some way important in the determination of defocus.

Spatial frequencies are differentially affected by defocus, defocus initially resulting in a loss of high spatial frequency information with lower frequencies being progressively affected as the level of defocus is increased. While contrast is also affected by defocus, resolution appears more sensitive to its effects. For example, visual acuity decreases by approximately 1 log unit with 2 to 2.5 D of uncorrected myopia (Hirsch, 1945) while approximately 7 D of uncorrected myopia is required to decrease contrast sensitivity by 1 log unit (Bradley *et al.*, 1991). Optical defocus has little effect on the contrast sensitivity for very low-spatial-frequency (i.e. large) stimuli but has a very large effect on high spatial frequency (i.e small) stimuli (Bradley *et al.*, 1991). If the defocus is sufficient to reduce spatial-frequency information to subthreshold levels then a uniform field would replace the patterned field perceptually. The influence of aberrations and defocus are most prominent for high spatial frequencies, although their effects remain, even at relatively low frequencies (Charman and Heron, 1979). However, given that the images of low-spatial-frequency gratings will remain perceptible for a greater magnitude of defocus than high-spatial-frequency gratings, could this differential effect of blur provide a defocus cue for the regulation of eye growth?

The chick, although possessing an area of retinal specialization, lacks a true fovea. An afoveate area centralis in the nasal retina has been

described (Morris, 1982), coinciding with the area of highest ganglion cell density reported by Ehrlich (1981). Based on ganglion cell densities the spatial resolution of the chick is estimated to be approximately 12.9 cycles/deg. This value is significantly higher than that reported in a behavioural study, i.e. 1.5 cycles/deg (Over and Moore, 1980). This resolution information was used in designing a study to examine the role of spatial frequency in emmetropization using restricted-spatial-frequency visual environments and an interrupted-occlusion paradigm.

6.2.2. Methods

Animals

Day-old male White Leghorn-New Hampshire cross chicks were used in this study. Chicks were reared under a 12 hr/12 hr light dark cycle, the light being provided by overhead fluorescent lights giving 250 lux at the level of the food trough. Food and water were provided *ad libitum* and the temperature kept at 30°C. All chicks were monocularly occluded for 10 days from hatching. Chicks were either constantly occluded or the occlusion treatment was interrupted with 20 mins of "visual stimulation" per day. During this period of "visual stimulation" chicks were exposed to either, i) a normal visual environment or ii) a restricted-spatial-frequency environment. In addition, a pilot study was undertaken to confirm that any myopic change produced by occlusion did not interfere with the initial visibility of the stimuli, 7 chicks were occluded from day 1, their refractive error measured on day 2 and their behavioural responses to the visual stimuli studied. The effect of defocus on the visibility of the spatial frequency stimuli was investigated by observing the effect of ±5 D, ±10 D and +15 D spectacle lenses.

Visual stimuli

Three different spatial frequencies were used; these were presented alone or were combined to give 5 different limited spatial frequency environments, the characteristics of which are summarized in Table 6.2.1. All consisted of high contrast (78%) vertical gratings, the only difference between the 5 stimulus conditions being their spatial frequency. While in the previous Chapter the intensities of the monochromatic lights were equalized on the basis of the spectral sensitivity curve of the chick, no

such attempt was made here to equalize the contrast of the different spatial frequency gratings on the basis of an undefined contrast sensitivity function for the chick; all gratings were generated at the maximum possible contrast.

The high-spatial-frequency stimulus (HSF) was a 4.3 cycles/deg square wave grating, falling between the reported behavioural acuity limit of the chick, 1.5 cycles/deg (Over and Moore, 1981), and the estimated anatomical limit of 12.9 cycles/deg (Ehrlich, 1981). The mid-spatial-frequency stimulus (MSF) was a 0.86 cycles/deg sine wave grating, slightly coarser than the behavioural acuity limit. The low-spatial-frequency stimulus (LSF) was a 0.086 cycles/deg sine wave grating. The mixed-spatial-frequency stimulus (MXSF) consisted of approximately equal amounts of the above three stimuli; a high/low stimulus (HLSF) was also generated by combining the high and low spatial frequencies. For technical reasons the high spatial frequency target, which required very closely spaced luminance changes, had to be generated as a square wave; this target included 4.3 cycles/deg sine wave and associated harmonic sine waves. The coarser targets were generated as sine wave gratings and contained only the specified spatial frequency information. In addition to avoiding the problem of contamination with higher order harmonics, the choice of sine waves meant that defocus effects could be easily predicted. In the case of sine waves, defocus simply reduces contrast without altering spatial frequency.

Table 6.2.1. Characteristics of restricted spatial frequency visual stimuli.

Visual stimuli	Spatial frequency (cycles/deg)	Grating type	Grating orientation
High (HSF)	4.3 and harmonics	Square wave	Vertical
Mid (MSF)	0.86	Sine wave	Vertical
Low (LSF)	0.086	Sine wave	Vertical
High/Low (HLSF)	4.3, 0.086	Square/Sine	Vertical
Mixed (MXSF)	4.3, 0.86, 0.086	Square/Sine	Vertical

All gratings were generated on a Macintosh computer at high contrast and printed using a phototypesetter to obtain high resolution prints.

These were scanned into the computer and intensity profiles plotted to ensure that sine waves had been produced as required. Prints were then laminated and attached to the inside of the "stimulus drum" via double sided velcro.

The normal visual stimuli (NV) which was included as a control, consisted of the chicks usual environment, i.e. 250 lux, other chicks, food, food containers.

Presentation of the visual stimuli

The apparatus described in section 6.1 to present limited-contrast environments was used here to present the restricted-spatial-frequency stimuli. In brief, the laminated stimuli were attached to the inside of a white, acrylic, cylindrical drum (50 cm diameter, 50 cm height; Plate 6.1). The drum was used to present the stimuli and could accommodate only one chick at a time. Chicks were placed in a round open container, their heads restrained via a neck brace, occluders were removed only after positioning in the drum. Chicks were exposed to the moving stimulus for 20 min. Afterwards the chick's occluder was replaced and the chick was removed from the drum and returned to its usual enclosure. In this manner, visual stimuli were consecutively presented to all chicks in any one batch.

The visibility of the stimuli was verified separately. The seven chicks assigned to this part of the study were placed individually into the drum; the speed of the drum was increased to 2.0 revs/min and the eyes of the chicks observed to determine if optokinetic nystagmus (OKN) could be elicited in response to the stimulus. This procedure was repeated for all three spatial frequencies, and on days 1, 2 and 3 after hatching.

In addition the effect of defocus on the visibility of the targets was investigated using ±5 D, ±10 D and +15 D spectacle lenses. A group of six chicks had one eye occluded at day 2 and the effect of the spectacle lenses, placed in turn in front of the normal eye, on the optokinetic nystagmus response to each of the three different spatial frequency stimuli observed.

Measurements

On days 5 and 10, refractive errors (on axis) and axial ocular dimensions were measured under halothane anaesthesia using retinoscopy (non-cycloplegic) and A-scan ultrasonography respectively. Using A-scan

ultrasonography, anterior chamber depth (ACD), axial lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL) data were obtained. In addition, to determine the presence or otherwise of relative asymmetry, the refractive errors of treated eyes and 10 randomly selected normal eyes were also measured off-axis at approximately 40 degrees into both the nasal and temporal visual fields again using retinoscopy. These eccentricities were the greatest possible for the maintenance of a clear, usable retinoscopic reflex. Corneal power (CP) was measured by infrared-video-photokeratometry under ketamine/Rhompun anaesthesia (see Appendix I for more details).

Chicks were then sacrificed using sodium pentobarbitone. The eyes were enucleated, cleared of extraneous muscle tissue and the external axial dimensions, i.e. axial length and equatorial diameters, measured directly with digital calipers. Eyes were also weighed on an electronic balance (see Appendix I for more details).

The 7 chicks assigned to the visibility study were refracted on day 2, at the time when the first period of visual stimulation was scheduled; this provided an estimate of the refractive error induced by occlusion and present at the commencement of the treatment.

Data analysis

Data were analyzed using nonparametric statistics. To test the difference between associated treated (T) and normal (N) eyes, the Wilcoxon matched-pairs signed-ranks test was used (WSRT). The Mann-Whitney U-test was used (MWUT) to assess the difference between different spatial frequency treatment groups; interocular differences (T-N) were compared in this case (see Appendix I for more details). Unless otherwise stated, all data are expressed as means ± SD in the results section.

6.2.3. Results

Visibility study

Refractions of the chicks on day 2, at the scheduled time of the first period of visual stimulation, gave a mean refractive error of +2.1±1.3 D for occluded eyes after 4.5 hrs of occlusion and +3.4±1.0 D for normal eyes. For occluded eyes refractive errors ranged from +4.0 D to –0.3 D (Fig. 6.2.1).

Figure 6.2.1. Refractive errors of treated and normal eyes at day 2, their ranges overlap.

Optokinetic nystagmus and following head movements were observed in 5/7, 7/7, 7/7 chicks at day 1 for the HSF, MSF and LSF stimuli respectively. These ratios increased to 7/7 for all treatment groups by day 2.

Table 6.2.2. Effect of defocus on optokinetic response.

	Spectacle lens (D)					
Visual stimuli	**–10**	**–5**	**0**	**+5**	**+10**	**+15**
High (HSF)	6 *	6 *	6 **	6 ***	6 ***	0
Mid (MSF)	6 *	6 *	6 **	6 ***	6 ***	5
Low (LSF)	6 *	6 *	6 **	6 ***	6 ***	2

*Number of chicks out of six for which an optokinetic response was observed. Stars represent the strength of responses with *** indicating the best and no stars the poorest responses.*

Optokinetic responses were observed in response to all the stimuli with all the spectacle lenses, the exception being the +15 D lenses (Table 6.2.2). Although responses were seen in all chicks, the strength and speed of the optokinetic response and following head movements increased with the

application of +5 and +10 D lenses and decreased for the –5 D and –10 D lenses. In contrast, responses with the +15 D lens were poor or absent and varied with the spatial frequency of the stimulus. No response to the high-spatial-frequency stimulus was seen in any of the six chicks, a third responded to the low-spatial frequency and the best response for the +15 D lenses was observed for the mid-spatial frequency.

Constant occlusion

Constant occlusion produced high myopia; mean refractive errors were –11.6±3.0 D ($P < 0.005$, WSRT) at day 5, increasing to –19.6±6.0 D ($P < 0.005$, WSRT) at day 10 (interocular differences, Table 6.2.3; see Appendix II, Tables AII.6.2, for treated and normal eye data). This myopia was primarily due to increased growth of the vitreous chamber and hence AL ($P < 0.005$, WSRT in all cases). Slight increases in ACD, 0.06±0.04 mm ($P < 0.05$, WSRT) and 0.09±0.08 mm ($P < 0.01$, WSRT), at days 5 and 10 respectively, also contributed to the observed axial changes. The enlarged corneas were also significantly steeper, at both days 5 and 10, ($P < 0.05$, WSRT in both cases). There was no significant effect of occlusion on ALT.

Table 6.2.3. Differences in ocular parameters between treated and normal eyes after 5 and 10 days of constant occlusion (mean ± SD; n = 7, both groups).

Ocular parameter	Day 5	Day 10
Δ Refraction (D)	–11.6±3.0***	–19.6±6.0***
Δ Corneal power (D)	+2.3±2.2*	+2.4±1.1*
Δ Anterior chamber depth (mm)	+0.06±0.04*	+0.09±0.08**
Δ Axial lens thickness (mm)	–0.002±0.01	–0.002±0.01
Δ Vitreous chamber depth (mm)	+0.46±0.09***	+0.76±0.25***
Δ Axial length (mm)	+0.51±0.16***	+0.87±0.3***

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test.*

Effect of daily periods of normal visual stimulation on form-deprivation myopia

Short daily periods of normal vision (NV) significantly reduced the magnitude of induced form-deprivation myopia (Table 6.2.4), from –11.6±3.0 D to –3.3±1.7 D after 5 days of treatment and from –19.6±6.0 D to –5.0±2.4 after 10 (Fig. 6.2.2, day 5; Fig. 6.2.4, day 10; see Appendix II, Table AII.6.2, for treated and normal eye data). This represents at decrease to 30% and 25% of CO levels at days 5 and 10 respectively. Consistent with observed differences in refractive error between the CO and NV groups, the latter group showed less increased vitreous chamber growth in response to occlusion (Fig. 6.2.3 and Fig. 6.2.5). Vitreous chamber elongation of 0.17±0.06 mm, i.e. 35% of CO levels and 0.29±0.18 mm, i.e. 40% of CO levels, were measured at day 5 and 10 respectively. Axial changes showed a similar pattern (Fig. 6.2.2, day 5; Fig. 6.2.4, day 10), 0.18±0.09 mm, i.e. 35% of CO levels, and 0.35±0.15 mm, i.e. 40% of CO levels, at days 5 and 10 respectively.

Increases in ACD produced by occlusion were also reduced when occlusion was interrupted by normal vision (Fig. 6.2.3, day 5; Fig. 6.2.5, day 10). The increased ACD of occluded eyes was decreased from 0.06±0.04 mm to 0.01±0.02 mm, i.e. 15% of CO levels, at day 5 and from 0.09±0.10 mm to 0.06±0.08 mm, i.e. 65% of CO levels, at day 10. The slight corneal steepening observed for CO was prevented when occlusion was interrupted by normal vision. ALT was unaffected by constant occlusion and there was also no effect of "interrupted occlusion" on lens thickness (Fig. 6.2.3, day 5; Fig. 6.2.5, day 10). Thus in percentage terms prevention of ACD changes was greater than prevention of VCD changes at day 5 and at day 10 the reverse was true.

Effect of short daily periods of visual stimulation with restricted spatial frequency environments on form-deprivation myopia

The ability of restricted-spatial-frequency environments to reduce occlusion-induced myopia varied with the spatial frequency presented, i.e. the results showed spatial-frequency dependence (see Appendix II, Tables AII.6.2, for treated and normal eye data). The MSF and MXSF environments were as good as the normal visual environment at preventing occlusion-induced myopia, with observed changes in refraction of –4.0±1.5 D (MSF, 35% CO value) and –4.1±1.2 D (MXSF, 35% CO value) respectively compared with –3.3±1.7 D (30% of CO value) for

normal vision, at day 5; equivalent values of –4.9±1.8 D (MSF, 25% of CO) and –4.9±2.1 D (MXSF, 25% of CO) compared with –5.0±2.4 D (NV, 25% of CO), were observed at day 10. There was no significant difference in occlusion-induced myopia for the HSF stimulus compared with that resulting from constant occlusion; a mean change in refraction of –10.8±3.0 D (95% of CO value) was observed for the HSF group compared with –11.6±3.0 D (CO), at day 5. There was a slightly greater but still small reduction in myopia for the HSF stimuli at day 10, i.e. to 70% CO levels. The LSF stimulus was also poor at preventing occlusion-induced myopia with changes in refraction of –7.4±2.1 D at day 5 (65% of CO level), and –8.7±5.2 D (45% of CO level) at day 10 being recorded. Mixing the HSF and LSF stimuli, i.e. the HLSF stimulus, was no more effective in inhibiting the development of myopia than each of these frequencies presented alone, with refractive changes of –7.4±3.1 D (65% of CO level) and –9.7±2.4 D (50% of CO level) being recorded at days 5 and 10 respectively.

As for normal vision, reductions in occlusion-induced myopia for short periods of visual stimulation with reduced spatial-frequency information reflected reduced occlusion-induced effects on anterior and vitreous chamber depths and hence axial eye dimensions. The restricted-spatial- frequency stimuli that were poor at preventing occlusion-induced myopia were also poor at preventing occlusion-induced increases in these ocular parameters. The converse was also true.

Deprivation-induced vitreous chamber elongation was greatly decreased from 0.46±0.09 mm to 0.17±0.04 mm (35% of CO level) and 0.22±0.08 mm (50% of CO level) with short daily visual stimulation with MSF and MXSF stimuli respectively at day 5; values at day 10 were 0.76±0.25 mm to 0.35±0.10 mm (MSF, 45% of CO level) and 0.28±0.11 mm (MXSF, 35% of CO level; Fig. 6.2.3, day 5; Fig. 6.2.5, day 10). In contrast, the HSF, LSF and combined HLSF stimuli were poor at preventing the increased growth of the vitreous chamber induced by occlusion, with the following values being recorded at day 5: 0.39±0.12 mm (HSF, 85% of CO level), 0.34±0.07 mm (LSF, 75% of CO level) and 0.35±0.13 mm (HLSF, 75% of CO level). At day 10 values were 0.58±0.17 mm (HSF, 75% CO), 0.56±0.16 mm (LSF, 75% of CO level) and 0.46±0.15 mm (HLSF, 60% CO).

The changes seen in AL mirrored those for VCD, with the MSF and MXSF stimuli being most effective at reducing the axial response to constant occlusion and the HSF, LSF and combined HLSF stimuli being relatively ineffective.

The deepening of the anterior chamber observed for occlusion was reduced by the introduction of short periods of visual stimulation and this reduction was also spatial-frequency dependent (Fig. 6.2.3, day 5; Fig. 6.2.5, day 10). Increases in ACD were reduced from 0.06±0.09 mm to –0.01±0.02 mm (0% of CO level) and 0.02±0.03 mm (35% of CO level) with short daily visual stimulation with MSF and MXSF respectively, at day 5 and decreased from +0.10±0.11 mm to 0.01±0.03 mm (10% of CO level) and 0.04±0.06 mm (40% of CO level) respectively, at day 10 (Fig. 6.2.3, day 5; Fig. 6.2.5, day 10). The HSF, LSF and combined HLSF stimuli were poor at preventing the deepening of the anterior chamber seen with form deprivation; indeed, visual stimulation with the HSF and HLSF stimuli resulted in anterior chamber deepening that was even greater than that produced by constant occlusion. Interocular ACD differences of 0.07±0.03 mm (HSF, > CO level), 0.03±0.03 mm (LSF, 50% of CO level) and 0.05 ±0.03 mm (HLSF, 50% of CO level) were observed for the HSF, LSF and combined HLSF stimuli respectively at day 5; equivalent values were 0.14±0.18 mm (HSF, > CO), 0.09±0.09 mm (LSF, 90% of CO level) and 0.10±0.11 mm (HLSF, = CO) at day 10.

Table 6.2.4. Effect of short periods of visual stimulation on occlusion-induced form-deprivation myopia. Differences in refraction of treated and normal eyes measured at days 5 and day 10 (mean ± SD, n).

	Δ Refraction (D)	
Treatment group	**Day 5**	**Day 10**
Constant occlusion	–11.6±3.0, 7	–19.6±6.0, 7
Normal vision	–3.3±1.7, 7***	–5.0±2.4, 7***
High spatial frequency	–10.8±3.0, 10•	–13.5±6.0, 9•••
Mid spatial frequency	–4.0±1.5, 10***	–4.9±1.8, 8***
Low spatial frequency	–7.4±2.1, 10**••	–8.7±5.2, 7**••
High/low spatial frequency	–7.4±3.1, 10**••	–9.7±2.4, 7**••
Mixed spatial frequency	–4.1±1.2, 10***	–4.9±2.1, 9***

*Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted-spatial-frequency groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005; Mann-Whitney U-test (one-tailed).*

The lens was unaffected by constant form deprivation and there was also no effect when periods of restricted-spatial-frequency stimulation were combined with occlusion at either days 5 or 10 (Fig. 6.2.3, day 5; Fig. 6.2.5, day 10).

Predicted changes in refractive error based on measured changes in ACD, VCD and corneal power were generally very similar to those measured using retinoscopy, at both days 5 and 10 (Table 6.2.5). Thus, where measured refractive errors were low so were predicted values. The converse was also true. This analysis also indicated that vitreous chamber elongation contributed most to the myopic shift seen in each of the treatment groups.

Table 6.2.5. Predicted (based on ocular parameter changes) compared with measured changes in refractive error (RE) for groups exposed to occlusion interrupted by restricted spatial frequency visual stimulation paradigms at days 5 and 10.

	Day 5					Day 10				
	HSF	**MSF**	**LSF**	**HLSF**	**MXSF**	**HSF**	**MSF**	**LSF**	**HLSF**	**MXSF**
Measured Δ RE (D)	–10.8	–4.0	–7.4	–7.4	–4.1	–13.5	–4.9	–8.7	–9.7	–4.9
Δ RE ACD (D)	–2.0	+0.3	–0.9	–1.5	–0.6	–4.1	–0.3	–2.6	–2.9	–1.2
Δ RE VCD (D)	–6.2	–2.7	–5.4	–5.6	–3.5	–9.2	–5.5	–8.9	–7.3	–4.4
Measured Δ CP (D)	+3.3	+0.2	+0.9	+0.7	–0.5	+2.9	–1.4	+0.3	+0.4	–0.9
Predicted Δ RE (D)	–11.5	–3.2	–7.2	–7.8	–3.6	–16.2	–4.4	–11.8	–10.6	–4.7

Based on schematic eye data of Schaeffel and Howland (1988a, see Appendix I for details).

Effect on ocular refractive symmetry

Refractive errors of treated eyes and 10 randomly selected normal eyes were measured both on-axis and approximately 40 degrees into the nasal and temporal fields using retinoscopy. Refractions measured in the nasal visual field were significantly more myopic (0.6 D to 0.8 D), than those measured in the temporal field (Table 6.2.6) for both normal eyes and treated eyes of all treatment groups. This was due to slightly less myopia in the temporal field compared with on-axis and slightly greater myopia

in the nasal field compared with on-axis but, while these were consistent findings, the differences were not significant. There were no significant treatment effect on the difference between nasal and temporal refractions.

External eye data

The trends in the ultrasound measures of axial length for the day 10 data were confirmed by external measurements of axial length (Fig. 6.2.7). The mean external axial length of constantly occluded eyes was 9.90±0.32 mm compared with 9.03±0.20 mm for contralateral normal eyes; these differences correspond to an average increase of 0.87±0.30 mm in axial eye growth in response to occlusion. The increase in external axial eye length was significantly less for all "interrupted-occlusion" groups, although consistent with the ultrasound data. The increase was least for MSF and MXSF groups and greatest for HSF, LSF and HLSF treatment groups.

Table 6.2.6. Refractive errors measured across the retina; on axis, in the nasal visual field and temporal visual field, at day 5 (mean ± SD, n).

	Refraction (D)		
Treatment group	**On axis**	**Nasal field**	**Temporal field**
Constant occlusion	–7.2±3.8, 7	–7.5±3.9, 7*	–6.7±3.9, 7
Normal eyes	+2.5±1.5, 10	+2.1±1.7, 10*	+2.6±1.4, 10
Normal vision	–1.1±1.9, 7	–1.5±1.8, 7*	–0.7±1.7, 7
High-spatial frequency	–7.5±4.0, 10	–7.8±3.9, 10*	–7.1±4.2, 10
Mid-spatial frequency	–0.7±1.0, 10	–1.2±0.8, 10*	–0.6±0.9, 10
Low-spatial frequency	–4.3±2.7, 10	–4.4±2.9, 10*	–3.8±3.1, 10
High/low-spatial frequency	–3.9±3.8, 10	–4.4±3.8, 10*	–3.8±4.0, 10
Mixed-spatial frequency	–0.8±1.86, 10	–1.1±1.7, 10***	–0.6±1.7, 10

*Differences between nasal and temporal visual field refractions significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test. The magnitude of the differences between nasal and temporal visual fields were not significantly different for different treatment groups Mann-Whitney U-test.*

In addition to causing axial expansion, constant occlusion also caused equatorial eye expansion. The mean equatorial diameter of constantly occluded chick eyes was 12.32±0.30 mm compared with 11.80±0.20 mm for contralateral normal eyes, corresponding to an average increase of 0.52±0.13 mm. All interrupted-occlusion paradigms decreased this effect

Constant occlusion also resulted in heavier eyes, i.e. an increase in wet eye weight. The wet eye weight of constantly occluded chick eyes was 0.71±0.06 g compared with 0.61±0.04 g for contralateral normal eyes, i.e. an average increase of 0.1±0.03 g. Here also, all "interrupted-occlusion" groups showed less effects of occlusion, an exception being the HSF group where increased eye weight represented 90% of the CO value.

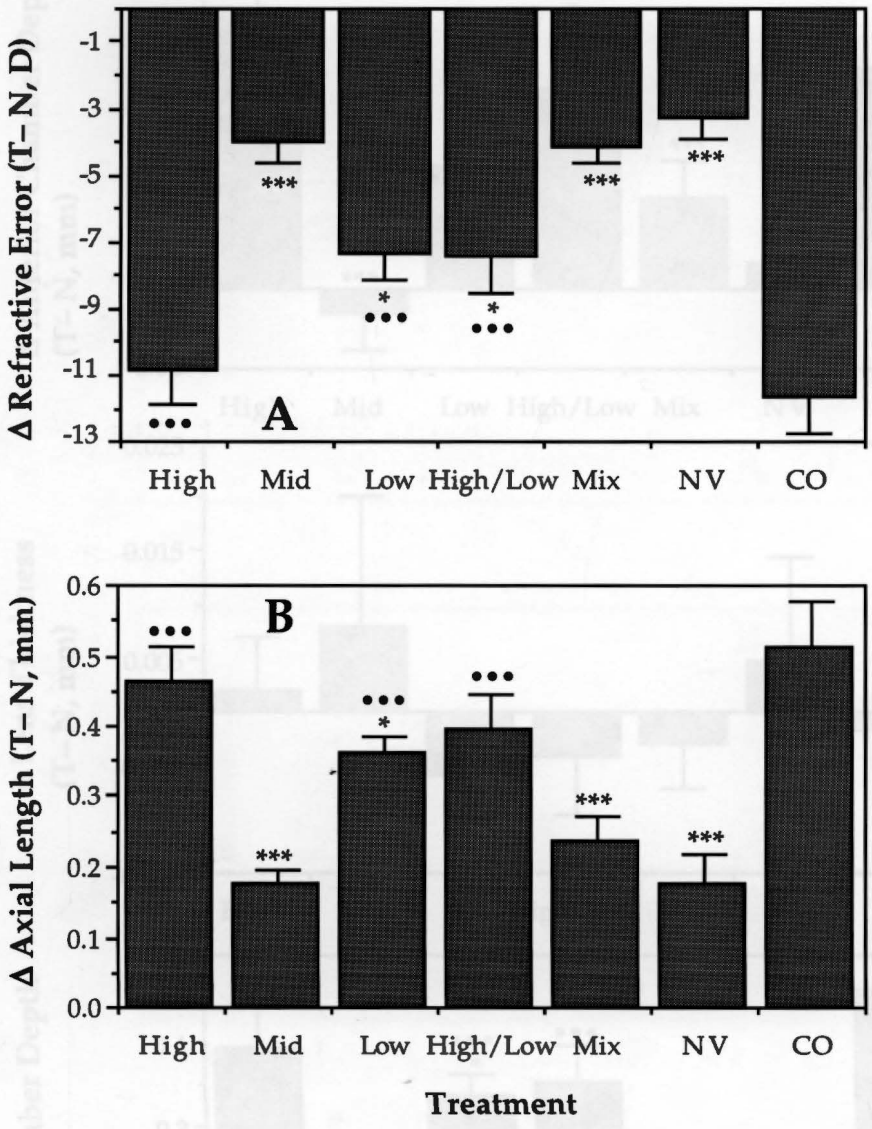


Figure 6.2.2. Differences (mean ± SE) at day 5, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted-spatial-frequency (High, Mid, Low, High/Low, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted-spatial-frequency groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

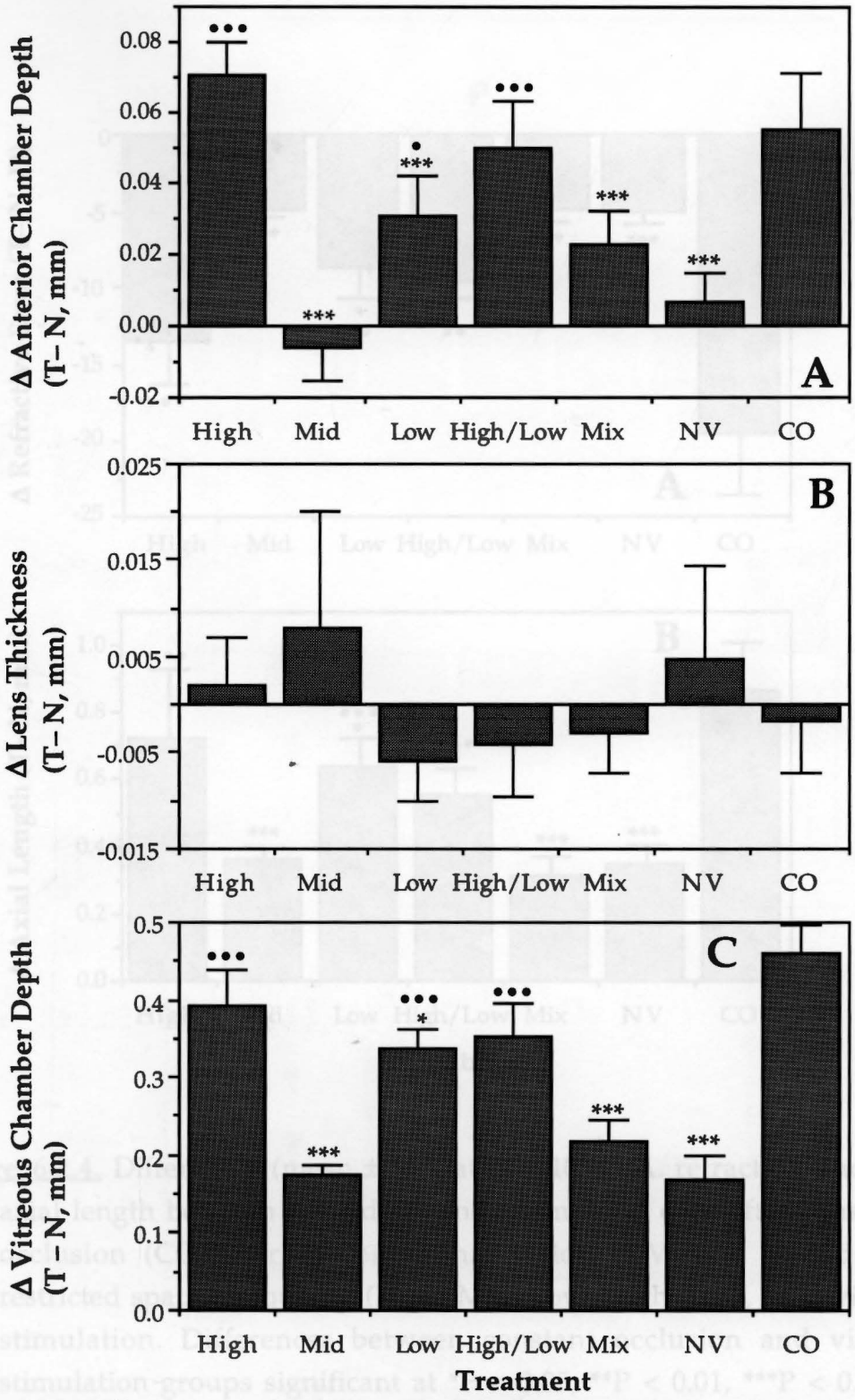


Figure 6.2.3. Differences (mean ± SE), at day 5, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted-spatial-frequency (High, Mid, Low, High/Low, Mix) visual stimulation. Differences between constant and interrupted occlusion groups significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, differences between normal vision and restricted-spatial-frequency groups significant at •$P < 0.05$, ••$P < 0.01$, •••$P < 0.005$, Mann-Whitney U-test (one-tailed).

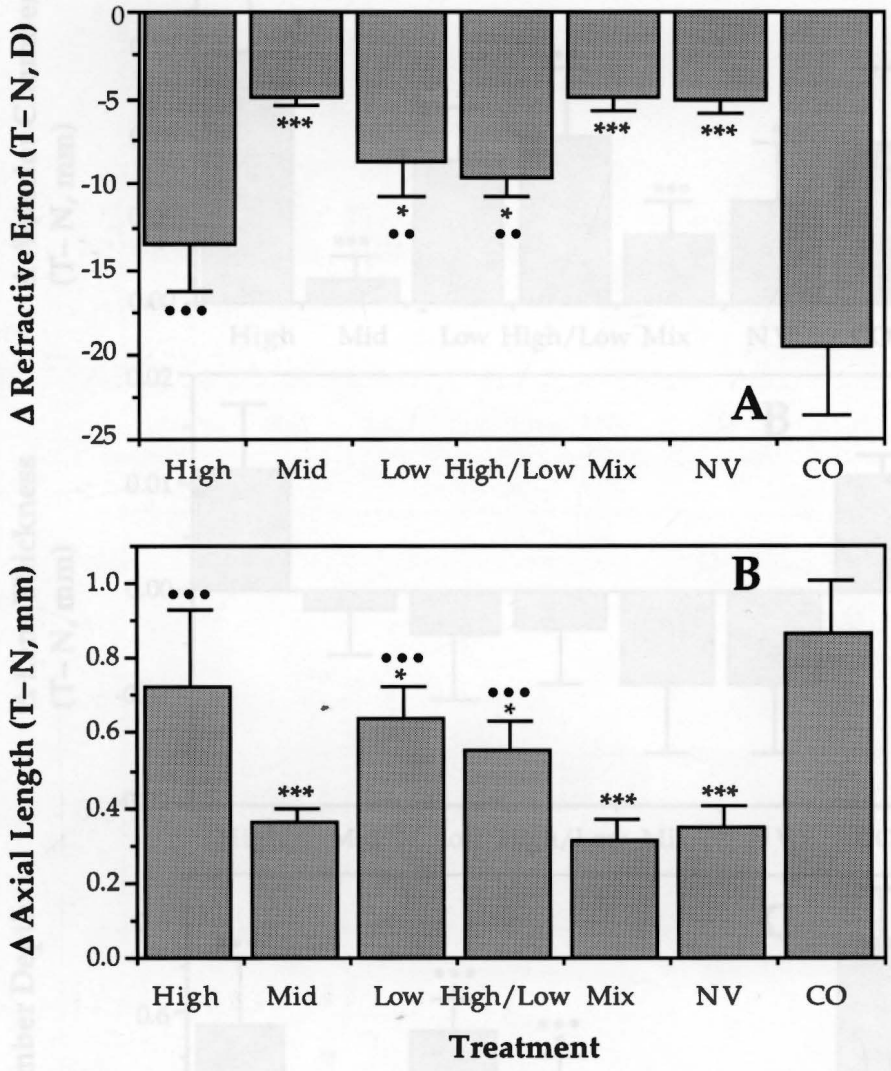


Figure 6.2.4. Differences (mean ± SE), at day 10, in **A.** refraction and **B.** axial length between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted spatial frequency (High, Mid, Low, High/Low, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted-spatial-frequency groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

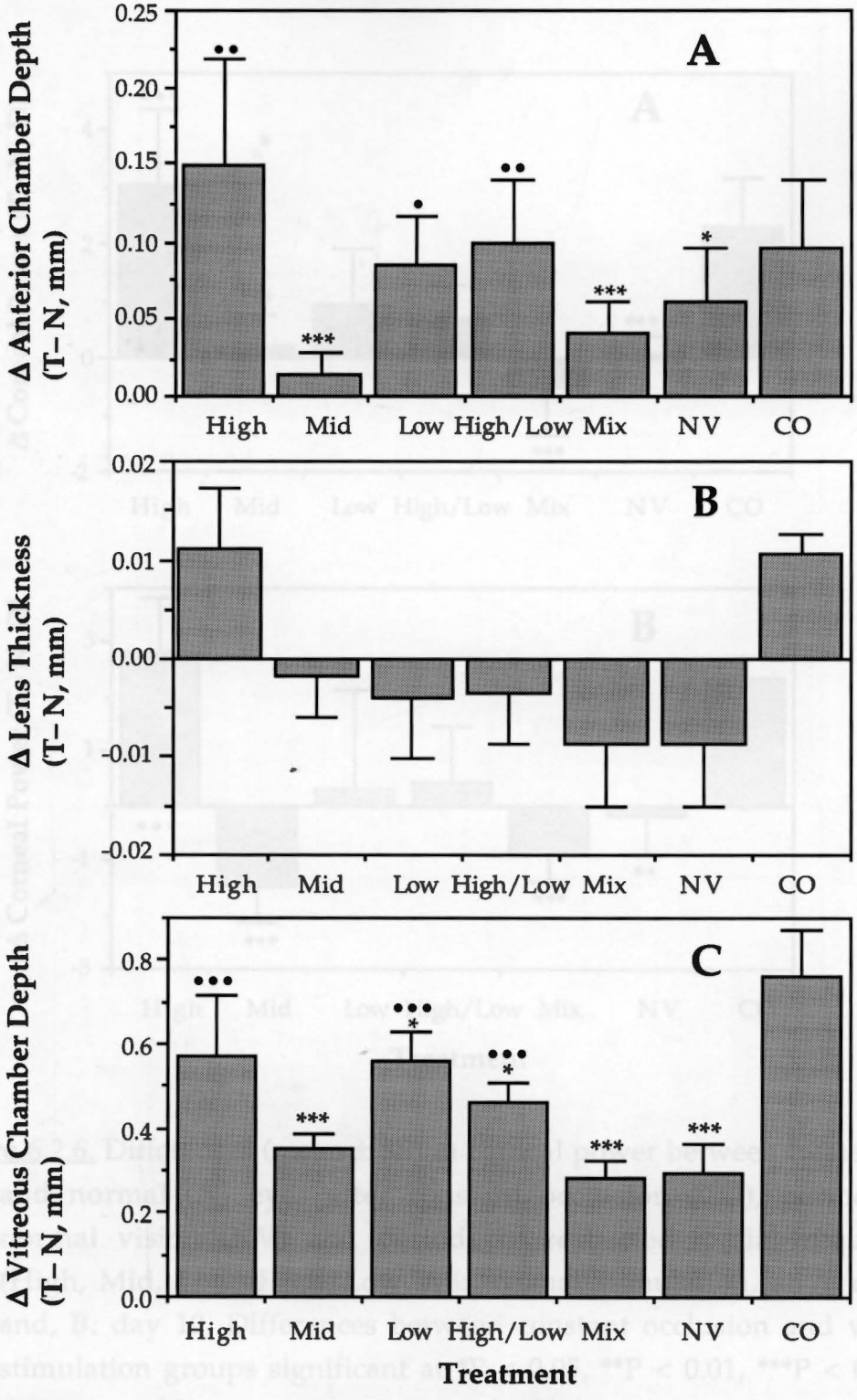


Figure 6.2.5. Differences (mean ± SE), at day 10, in **A.** anterior chamber depth, **B.** lens thickness and **C.** vitreous chamber depth between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted-spatial-frequency (High, Mid, Low, High/Low, Mix) visual stimulation. Differences between constant and interrupted occlusion groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted-spatial-frequency groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

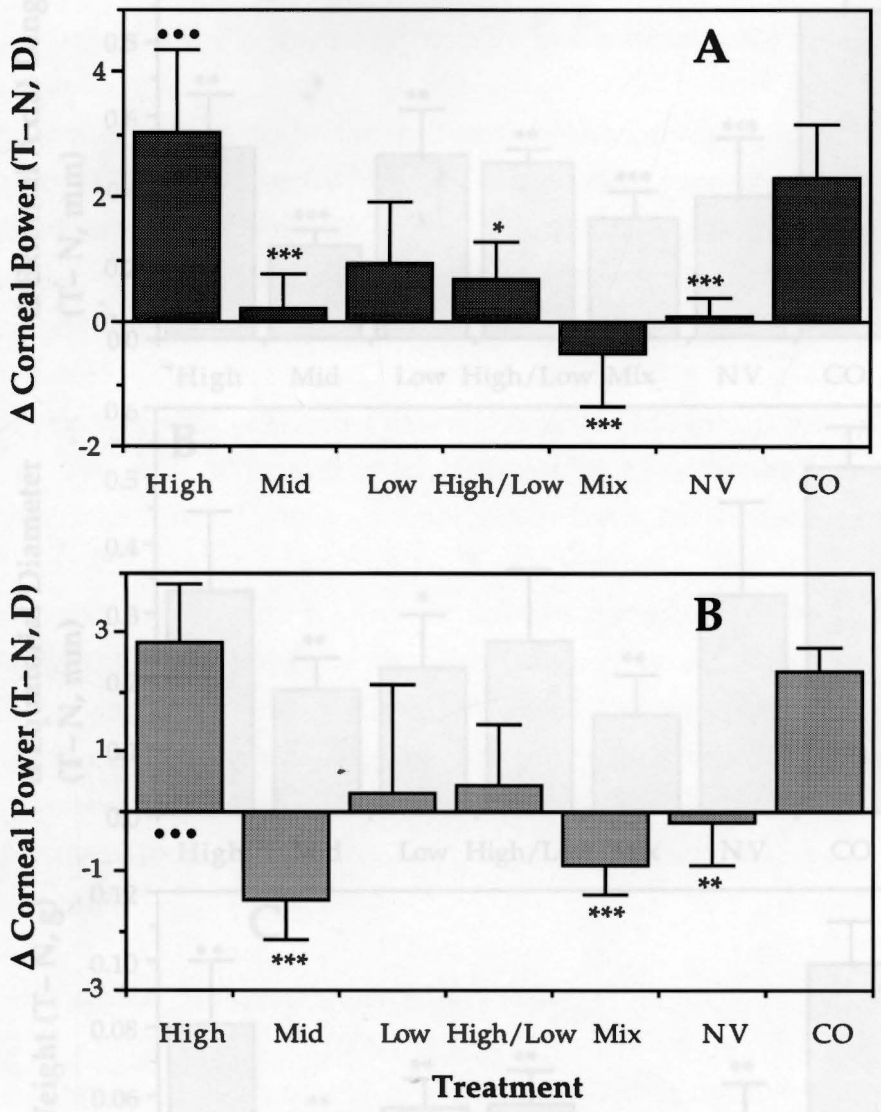


Figure 6.2.6. Differences (mean ± SE) in corneal power between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted-spatial-frequency (High, Mid, Low, High/Low, Mix) visual stimulation, at **A.** day 5 and, **B.** day 10. Differences between constant occlusion and visual stimulation groups significant at *P < 0.05, **P < 0.01, ***P < 0.005, differences between normal vision and restricted-spatial-frequency groups significant at •P < 0.05, ••P < 0.01, •••P < 0.005, Mann-Whitney U-test (one-tailed).

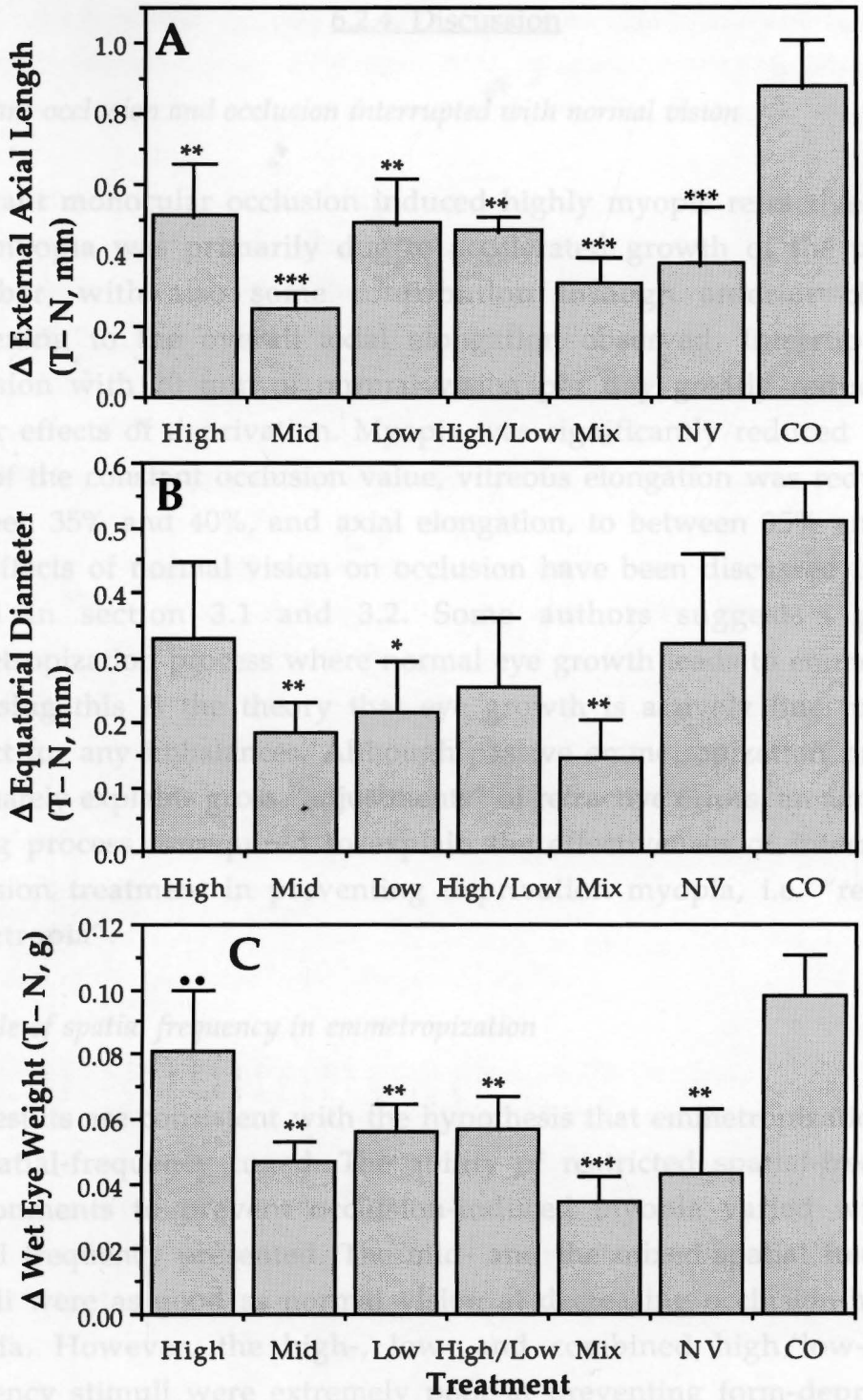


Figure 6.2.7. Differences (mean ± SE), at day 10, in **A.** external axial length, **B.** equatorial diameter and **C.** wet eye weight between treated (T) and normal (N) eyes after constant occlusion (CO), periods of normal vision (NV) and periods of restricted-spatial-frequency (High, Mid, Low, High/Low, Mix) visual stimulation. Differences between constant occlusion and visual stimulation groups significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, differences between normal vision and restricted-spatial-frequency groups significant at •$P < 0.05$, ••$P < 0.01$, •••$P < 0.005$; Mann-Whitney U-test (one-tailed).

6.2.4. Discussion

Constant occlusion and occlusion interrupted with normal vision

Constant monocular occlusion induced highly myopic refractive errors. The myopia was primarily due to accelerated growth of the vitreous chamber, with also some contribution through anterior chamber deepening to the overall axial elongation observed. Interruption of occlusion with 20 min of normal vision per day greatly reduced the ocular effects of deprivation. Myopia was significantly reduced to only 30% of the constant occlusion value, vitreous elongation was reduced to between 35% and 40%, and axial elongation, to between 35% and 40%. The effects of normal vision on occlusion have been discussed in more detail in section 3.1 and 3.2. Some authors suggest a passive emmetropization process where normal eye growth leads to emmetropia. Opposing this is the theory that eye growth is actively fine tuned to correct for any imbalances. Although passive emmetropization probably adequately explains gross "adjustments" of refractive errors, an active fine tuning process is required to explain the effectiveness of interrupting occlusion treatment in preventing deprivation myopia, i.e. "restoring emmetropia".

The role of spatial frequency in emmetropization

The results are consistent with the hypothesis that emmetropization may be spatial-frequency tuned. The ability of restricted spatial-frequency environments to prevent occlusion-induced myopia varied with the spatial frequency presented. The mid- and the mixed-spatial frequency stimuli were as good as normal vision at decreasing occlusion-induced myopia. However, the high-, low- and combined high/low-spatial frequency stimuli were extremely poor at preventing form-deprivation myopia. Indeed, there was no significant difference in the magnitude of occlusion-induced myopia with high-spatial frequency stimulation compared with that resulting from constant occlusion. Mixing the high- and the low-spatial frequency stimuli did not provide a stimulus that was any better at preventing the response to occlusion than either stimulus presented alone. The data suggest that emmetropization is spatial frequency dependent; moreover mid-frequency spatial information, but not high- or low-frequency spatial information, can be used in some way

to prevent form-deprivation myopia. These results also imply, although they provide no direct evidence, that intermediate spatial-frequency information provides information about defocus that can be used in emmetropization.

It has been suggested that accommodative responses are more accurate for square-wave stimuli than for simple sine-waves of the same fundamental frequency (Charman and Tucker, 1977; Charman and Tucker, 1978b; Ciuffreda *et al.*, 1987). This suggests that a "rich" mixture of spatial-frequency information provides better access to defocus information. By analogy, one might expect that emmetropization would similarly be more accurate to a square-wave or varied frequency stimulus. Thus a mixed-spatial frequency stimulus would be more effective at preventing form-deprivation myopia than a mid-spatial frequency stimulus alone and possibly, although less likely, that a combined high/low-spatial frequency stimulus would be better than a high-spatial frequency or low-spatial frequency stimulus alone. These predictions were not supported by the data; the mid- and mixed-spatial frequency stimuli were equally effective stimuli for emmetropization, and the individual and combined high- and low-spatial frequency stimuli were equally poor.

Resolution of the restricted spatial-frequency stimuli

As an explanation for the minimal effect of the high-spatial frequency stimulus on the occlusion response, it could be argued that as this high-spatial frequency stimulus is finer than the reported behavioural acuity limit of the chick (Over and Moore, 1981) and thus was not able to be resolved. Under these circumstances, it would appear as a uniform field to the chick and thus essentially no different from the constant-occlusion condition. However, this possibility can be ruled out by three factors. Firstly, the myopic shift produced by day 2 when the visual stimuli were first presented, was extremely low (1.3 D). As all chicks were initially hyperopic, this change in refraction meant that only 6 D to 7 D of accommodation was required to view the target at 25 cm. This task is well within the accommodative capacity of the young chick (Schaeffel *et al.*, 1986; Troilo and Wallman, 1987; section 4.2). Secondly, while it could also be argued that the anisometropia induced would result in the occluded eye being 1 D out of focus if the normal eye fixated, accommodation in the

chick is not consensually linked (Schaeffel *et al.*, 1986) and thus accurate focus for the targets by each eye simultaneously can be assumed. Finally, good optokinetic nystagmus and pursuit head movements were generally elicited for all targets. Although these responses were seen in only 5 out of 7 chicks for the high-spatial frequency target at day 1, all chicks responded to this target by day 2. It was assumed that, as this response is visually mediated, a positive response indicated that the chicks could "see", i.e. resolve the target.

It could also be argued that although the high-spatial-frequency target was initially visible it might become subthreshold with time due to the effects of deprivation-induced myopia. When it would then appear as an empty field. However, this effect is unlikely as significant amounts of defocus, i.e. +15 D, was required to affect the optokinetic response to the high-spatial-frequency stimulus. For lower amounts of defocus, i.e. +5 D and +10 D, the response to the target was faster and more obvious than normal with the defocussing lenses; this is presumably due to the positive lenses magnifying the stimuli and thus rendering them more visible.

Based on these optokinetic nystagmus responses, one is also forced to conclude that the behavioural spatial acuity of the chick is higher than previously estimated being at least 4 cycles/deg. This value is still less than that of 12.9 cycles/deg estimated from anatomical means (Ehrlich, 1981) but is much higher than the previously reported behavioural acuity limit of 1.5 cycles/deg (Over and Moore, 1981). It should also be noted that the optokinetic nystagmus pathway, at least for mammals, has a lower resolution capacity than seen with static targets. This difference supports the argument that behavioural values are too low and that the chicks were able to "see" the high-spatial frequency stimulus.

While some of the improvement in the optokinetic response with low powered positive lenses can be explained in terms of the lenses providing a refractive correction and thus presumably "clearer" image (the chicks were hyperopic at the time), most of the improvement in response seen with positive lenses and also the decrease with negative lenses is probably due to changes in magnification, with the positive lenses increasing and the negative lenses decreasing retinal image size. This would also contribute to the faster optokinetic response for positive lenses and the relatively slower response with negative lenses. An analogous situation has been reported in primates where by altering the

visual environment with magnifying lenses, saccades, the vestibulo-ocular reflex and optokinetic nystagmus are modified (Miles and Fuller, 1974; Miles and Eighmy, 1980). Wallman *et al.* (1978a) have reported the loss of optokinetic response in chicks wearing translucent occluders and its restoration when a small hole is made in the occluder (1.8 mm); this reinforces the fact that this response is a visual one.

It should also be noted here that the different spatial frequency gratings were not equalized for visibility on the basis of contrast sensitivity as this function for the chick is not currently known. However, as all gratings were generated at high contrast and theoretically should have been well above the contrast threshold for detection, only a minimal effect due to this difference is likely. It could also be argued that the way the targets were presented here is more representative of a normal environment where high contrast stimuli at all frequencies are usually present. None the less it should be noted that there was a slight difference in visibility of the stimuli as reflected in the effect of spectacle-lens-induced defocus on the behavioural response to the stimuli; the response to the high-spatial-frequency stimulus was most affected by the +15 D lens and the response to intermediate-spatial-frequency stimulus least affected.

Why aren't high spatial frequencies good emmetropization stimuli?

The result that high spatial frequencies alone are poor at guiding emmetropization is not entirely surprising, as there is much indirect evidence which suggests that this may be the case. This would also make sense as a very small focussing error will attenuate or eliminate high-spatial-frequency information, unless pupils are extremely small and there is a very large depth of focus. For example, a large amount of monocular optical defocus is required to disrupt the emmetropization process of kittens (Nathan *et al.*, 1984). As defocus acts as a high-spatial-frequency filter and it is believed that kittens don't accommodate over the lenses, it is likely that their emmetropization mechanisms do not require high-spatial-frequency information and/or high image contrast. Emmetropization also occurs under relatively dim, i.e. low luminance conditions (section 5.3); if the emmetropization process relied on high spatial frequencies and thus high resolution pathways only, emmetropization should have been much poorer under such conditions.

Reports that the visual systems of humans and monkeys are immature at birth, are incompatible with a high-spatial-frequency guided emmetropization process. Visual resolution in the monkey is poor until long after birth (Jacobs and Blackemore, 1988). Similarly, the contrast sensitivity of human infants is lower than that of adults by nearly two log units at all frequencies and infants are only sensitive to low frequencies, i.e. below about 3 cycles/deg. Thus at one month an infant can see no fine details and can only see relatively high contrast, large objects (Banks, 1982/83). It takes approximately six months for the contrast sensitivity function and acuity to reach near adult levels (Pirchio *et al.*, 1978; Norcia and Tyler, 1985), although behavioural acuity measured by preferential looking techniques is still poor (Dobson and Teller, 1978). It could thus be argued that it would make "functional sense" to avoid a dependence on high spatial frequencies, at least during early development, to allow early tuning of refractive error.

Comparison to the human accommodation system

A recent study by Stone *et al.* (1993) found that longitudinal chromatic aberration was a fundamental cue to reflexive accommodation and that the magnitude of longitudinal chromatic aberration was optimal for intermediate spatial frequencies between 3 and 5 cycles/deg. This is also the range of frequencies for which accommodation is most accurate and at 3 cycles/deg, for moderate pupil sizes, longitudinal chromatic aberration is postulated to be the principal cause of retinal blur. Thus, if longitudinal chromatic aberration is used by the human visual system to detect accommodative defocus, it could also be used by the chick system to detect defocus during ocular growth. This cue may be optimal around 1 cycles/deg for the chick, where emmetropization is most accurate. However, while longitudinal chromatic aberration may be used under normal lighting conditions to guide emmetropization, monochromatic light studies in chicks (Wildsoet *et al.*, 1993) show that, when longitudinal chromatic aberration is unavailable as a cue, an alternative non-chromatic cue (or cues) is used to guide emmetropization (section 5). The results presented here argue that this, as yet unknown, non-chromatic cue is similarly spatial-frequency-dependent.

Consistent with the finding that low-spatial-frequency gratings provide a poor stimulus for emmetropization in the chick, it has been

shown in humans that accommodative responses are inaccurate when low spatial frequency sinusoidal gratings are used as targets (Charman and Tucker, 1977; Charman and Tucker, 1978b; Owens, 1980). Although still an issue of contention, it has been suggested that intermediate spatial frequencies, near the peak of the contrast sensitivity function, are the most important for the human accommodation system and that a high-spatial-frequency content is not necessary for accurate responses (Owens, 1980). The peak of the contrast sensitivity function of the pigeon is about 2 cycles/deg, spatial frequencies around this peak may be the most important for emmetropization. The increased accuracy of emmetropization in chicks to intermediate-spatial-frequencies suggests that they use a similar approach to defocus detection.

Significance of ocular symmetry study

In many vertebrates, the density of retinal cells varies dramatically across the retina. Regions with increased spatial density of photoreceptors and ganglion cells possess higher visual acuity compared with less cell dense regions. Foveal or central acuity is usually much higher than that in the periphery. Unlike many diurnal birds which have well-developed foveae, (and in some cases, two foveae), the chick, although possessing an area of retinal specialization, lacks a true fovea. The chick retina has a relatively uniform ganglion cell density with only an approximate 6-fold change across the retina (least dense in periphery; Ehrlich, 1981) and corresponding 2-fold decrease in acuity. Based on this decrease in ganglion cell density, the spatial resolution is estimated to fall from approximately 12.9 cycles/deg to 9.1 cycles/deg in the mid-periphery and to 5.3 cycles/deg in the extreme periphery. If emmetropization is tuned to the "highest resolvable frequency" then, based on these resolution limits, it could be expected that the high-spatial frequency target used here would be a good, critical visual stimulus for the central retina and the mid- and low-spatial frequency for the peripheral retina. The initial hypothesis was that the high-spatial frequency stimulus would result in less myopia centrally than peripherally and conversely the mid- and low-spatial frequency stimuli would result in less myopia peripherally compared with centrally. Results were not as predicted; refractive gradients, with slightly greater myopia in the nasal field compared with central and temporal fields, were observed for all treatment groups. However, the lack of a large centro-peripheral gradient is perhaps not surprising considering the relative uniformity of the chick retina and the fact that the mid-spatial frequencies were found to be the most important for emmetropization.

When high myopia was measured axially, it was also measured in the periphery and likewise when low myopia was measured axially only low myopia was measured in the periphery. This trend is consistent with that reported in section 6.1 where variable contrast stimuli were used in the same paradigm and would suggest that this is an anatomical rather than visually determined gradient (at least in the context of this experiment).

Significance for myopia due to visual deprivation

Lid fusion, in monkey and cat, has been shown to have three major effects on the retinal image: reduced retinal illumination, decreased image contrast at all spatial frequencies, particularly high spatial frequencies, and altered spectral composition of the image (Crawford and Marc, 1976; Loop and Sherman, 1977). Similarly in the chick, occlusion has been shown to decrease the contrast and affect the spatial frequency distribution of the image (Hodos and Kuenzel, 1984). Emmetropization still occurs when chicks are reared under relatively dim light conditions (section 5.2) and under monochromatic light (Wildsoet and Howland, 1991) ruling out the decrease in retinal illumination and altered spectral composition of the light as being the trigger for lid-suture myopia. Thus degradation of spatial vision, associated with the reduced image contrast and/or loss of high spatial frequency information, appears to be the most likely aspect of lid closure or occlusion that initiates the onset of myopia. The results of the current study would suggest that the loss of mid-spatial frequency information may be more important and thus occluders which only transmit low spatial frequencies will be more effective at inducing myopia, low-spatial frequency information when presented alone being poor at guiding emmetropization. Consistent with this prediction, myopia is not observed in chicks deprived using almost clear occluders, which presumably only affect very high spatial frequencies. Refractive errors of occluded eyes are on average +1.0±2.2 D hyperopic compared to normal eyes (n=6).

Significance for near-work myopia

The observation that form deprivation myopia is attenuated by intermediate spatial frequencies and not high spatial frequencies may have implications for the proposal of Wallman *et al.* (1987) linking "near-work myopia" and the high-spatial-frequency nature of printed text. They suggested that the high-spatial frequencies in text provided inadequate stimulation for non-foveal neurons with large receptive fields. In the current study, the high-spatial-frequency target did not provide an adequate stimulus for emmetropization; alternatively the high-frequency stimulus

may have itself generated an aberrant error signal. The latter alternative, albeit less attractive, cannot be ruled out at this point.

A discussion of the retinal pathway involved

The question of how spatial information in the retinal image is processed and transmitted is a complicated one. How it provides information about defocus is more complicated still. Studies in chick would suggest that the defocus signal must be detectable and hence processed by the retina (reviewed in Wallman, 1991 and 1993). Much image processing does occur in the retina before the visual signal leaves the eye. Although complicated information processing, e.g. retinal ganglion cell responses are orientation dependent, has been suggested as a feature of the avian retina (King-Smith, 1971), there are presently no clear indication as to which cells play the major role in visual resolution. Hence which retinal cells may detect defocus remains unknown.

Ikeda and Wright (1972) have shown that central retinal ganglion cells are very sensitive to defocus and require a sharply focussed image for excitation; peripheral cells are less sensitive to defocus. However data in chick would suggest that normal ganglion cell activity is not required for emmetropization responses (Wildsoet and Pettigrew, 1987). It has been suggested that amacrine cells are the most likely retinal cells for detecting defocus (reviewed in Wallman, 1991 and 1993). Unfortunately very little research has been conducted into the response properties of amacrine cells in general and none into the response properties of chick amacrine cells; this is partly due to the inherent difficulties associated with obtaining physiological responses from these cells which are located deep within the retina.

While the growth control mechanism in chick appears to be located largely within the eye, refractive errors of eyes following optic-nerve section tend to overshoot emmetropia, perhaps suggesting that feedback from the brain has some role in this process (Troilo and Wallman, 1987). Thus also of relevance here may be the fact that there are spatial frequency channels in the visual system that are sensitive to narrow ranges of spatial frequencies. Simple cortical cells in the cat have been shown to possess the properties required for analysis of spatial frequency information and differentially respond to different frequencies (Maffei and Fiorentini, 1973). Alternately, the loss of ganglion cells following optic nerve section may alter the spatial frequency sensitivity of the retina and this could contribute to the poorer emmetropization response.

"Transient" theory for myopia reduction

As described in the previous section for occlusion interrupted with restricted contrast stimuli, an analysis of retinal "transients" could explain the decrease or lack of decrease in myopia seen when occlusion is interrupted with the restricted spatial frequency stimuli. The visual system responds well to changing stimuli but responds poorly, or not at all, to constant stimuli. By dampening contrast, occluders decrease the number of "transients", i.e. the information received both before and after an eye movement are near to identical (reviewed in Wallman, 1991). During normal vision, moving edges would elicit transient responses. This could explain some of the lessening of myopia seen but predicts that the high rather then mid frequencies would have the greatest preventative effect.

Effect of temporal frequency

The targets provide different temporal frequency information which could contribute in part to the spatial-frequency-dependence observed. It is possible that, as form deprivation is sensitive to strobe (Gottlieb *et al.*, 1987; Squires *et al.*, 1992), even when presented over short durations (1 hr per day of 20 Hz strobe can reduce myopia to only –5 D after 1 week of treatment; Nickla, personal communication), observed reductions in myopia were due to an inhibitory strobe effect rather than to spatially-tuned emmetropization. If the chicks "stared" straight ahead at the stimuli then the stimuli would provide temporal frequencies of 26.3 Hz (HSF), 5.1 Hz (MSF) and 0.51 Hz (LSF). The optimal strobe effect occurs at about 10 Hz and falls at frequencies higher and lower than this (Squires *et al.*, 1992); this effect predicts a similar pattern of results to that observed. However, the chicks' heads were not restrained during testing and the chicks exhibited following head and eye movements for greater than half the time, during which the target would be stable on the retina, thus reducing any strobe effect. The only way to clarify this issue would be to investigate the effect of 20 min of flicker at rates equivalent to that produced by each of the stimuli.

6.2.5. Conclusion

The ability of restricted-spatial-frequency environments to reduce occlusion-induced myopia varied with the spatial frequency presented. The mid- and mixed-spatial frequency stimuli were as good as normal vision at decreasing occlusion-induced myopia. The high- and low-spatial frequency stimuli, whether presented alone or combined were poor at preventing occlusion-induced myopia. The data indicate that mid-spatial frequencies are required for emmetropization and that emmetropization is spatial frequency dependent.

CHAPTER 7

CONCLUSIONS, THE REFRACTIVE ERROR SIGNAL AND UNRESOLVED ISSUES

7.0. Conclusions, the Refractive Error Signal and Unresolved Issues

This Chapter summarizes the main findings of the experimental studies in this thesis and suggests a possible refractive error signal for the emmetropization system. Possible future experiments that could be performed to increase the knowledge of this system and unresolved issues are also discussed.

7.1. Summary and Implications of Results

The effects of removing or restricting one aspect of the visual image or one visual behaviour that may be important for emmetropization was studied; it must be emphasized that no attempt was made to control other aspects of the environment. Accommodation, chromatic aberration, contrast and spatial frequency were separately manipulated. The results suggest that when accommodation and chromatic aberration are eliminated other defocus cues can be or are used for emmetropization. The results do not rule out the possiblity that these cues may be used under circumstances of reduced visual information. The results argue that each of the cues tested is not the only cue used by the emmetropization system. An alternative way of describing this outcome is that there are multiple cues for defocus. The result is perhaps not surprising as the presence of multiple cues would make the emmetropization system potentially more reliable and accurate than if it relied solely on the information presented by one cue. This also means that if there are two or more alternative cues, both would need be eliminated to impair emmetropization.

The effect of manipulating contrast and spatial frequency on emmetropization in the chick shows surprising similarity to results obtained for the human accommodation system. Emmetropization is not dependent on a varied contrast environment and the results would suggest a threshold below which the emmetropization system is unable to

function. The information used for emmetropization is spatial frequency dependent, with intermediate frequencies being most important.

While it is not claimed that actual quantitative data can be extrapolated to the primate or human visual systems, based on the eye growth studies in primate to date that generally support chick data, general trends may be extrapolated as long as physiological differences, e.g. acuity, are taken into account. Thus emmetropization in humans may be similarly spatial frequency dependent but the frequencies most important for humans are likely to be higher than those for chicks, i.e. higher than 0.8 cycles/deg and similarly, while the presence of a contrast threshold for emmetropization is likely, it is expected to be much lower than that for the chick.

All of the results described above were determined for one breed of chick, the White Leghorn-New Hampshire cross. While there were subtle differences in the responses of different breeds of chicks (see Chapter 2) the major effects, i.e. high myopia in response to deprivation and recovery on restoration of vision were observed for both breeds. This gives credence to the suggestion that the major characteristics of emmetropization are not breed dependent. It is tempting to draw an analogy here, just like for human data where there are variations in the responses of individual subjects but average data are reported, a similar effect is observed for chicks.

7.2. Does longitudinal chromatic aberration and/or luminance contrast guide ocular growth?

A model is proposed for the refractive error defocus signal used during growth. It must be emphasized that while this is based on the results of this thesis, it is also somewhat speculative. The model predicts that the error detector derives information as to the direction and magnitude of the refractive error, by use of the information provided by the longitudinal chromatic aberration of the eye and by retinal image contrast. When refractive errors are extremely high, contrast is the most useful cue and when low, longitudinal chromatic aberration acts to fine tune the refractive state. This is of course only one model and others involving accommodation or multiple non-chromatic cues are possible.

Longitudinal chromatic aberration and contrast

The results of this thesis support the view, that there must be at least two different retinal blur detecting systems in the chick eye controlling ocular growth, the most likely being a chromatic system and a non-chromatic one, the chromatic system using longitudinal chromatic aberration and the non-chromatic system involving contrast. Although not providing concrete evidence, none of the results of this study are in conflict with this hypothesis and this model can be used to explain all of the results reported here. The existence of multiple visual cues is supported by data for human accommodation. There are widely differing accommodative strategies used by subjects given identical focussing tasks, for example, the vergence of light is a good accommodative stimulus for some subjects, but not others, and some subjects, but not all, use chromatic aberration for guiding focus (Kergoat and Lovasik, 1990).

The different contrast levels produced by longitudinal chromatic aberration in the different retinal colour opponent channels provide a cue as to the direction of defocus (Fig. 7.1). When small amounts of retinal defocus are present, contrast gradient information between different colour opponent channels determines the error signal; when large amounts of retinal defocus are present the mean contrast level controls the error response. The model can explain normal emmetropization, for example, when a low hyperopic refractive error is present the retinal image contrast of blue light will be greater than that of red light and this will indicate a hyperopic defocus and generate an "go signal". Conversely for low myopic refractive errors retinal image contrast of red light will be greatest, indicating myopia and initiating a "stop signal". Chromatic aberration generates opposite direction contrast gradients, for opposite directions of defocus. There are certain cells in the retina and LGN that respond to light at one end of the spectrum by increasing their firing rate and where light at the opposite end results in an inhibiton of spontaneous activity. In this way neural activity is transformed in a way that potentially enhances the visual sytem's ability to distinguish between different wavelengths (Schiller, Logothetis and Charles, 1990). When refractive errors are high this system loses its effectiveness as there will be negligable differences in the quality of red and blue images and the eye simply uses the average retinal image contrast to determine growth. For high myopia the retinal contrast varies, being greater for near objects; for high hyperopia, image contrast is reduced at all distances.

The results of form-deprivation, both constant and intermittent, can also be explained. In form-deprivation, the lid or occluder produces an image of extremely low contrast at all apparent viewing distances, which is misinterpreted as high hyperopia. This assumption about the type of refractive error is made on the basis that if the eye was myopic, the retina would receive a higher contrast image when viewing near targets; as the contrast of the image does not alter in form deprivation, this is similar to an extremely high hyperopic refractive error, i.e. blur at all distances, and an increase ocular growth signal is activated. Thus in this model, a large temporal variation in contrast indicates myopia and a constant, relatively unchanging contrast level indicates hyperopia. Retinal image contrast has also recently been suggested by Bartmann and Schaeffel (1994) to be used as a defocus cue during emmetropization. Increased growth continues unchecked in the case of form deprivation as the control system is open-looped, i.e. no alteration is retinal image contrast occurs as a consequence of growth. However, when the deprivation stimulus is removed, the eye receives a higher contrast signal when viewing near targets and a "stop signal" is initiated. With intermittent deprivation, the period of deprivation indicates a high hyperopic refractive error and the period of normal vision indicates myopia, the magnitude of form-deprivation-myopia is thus seen to be less when periods of normal vision are given. These differences in image contrast will be largely unaffected by the elimination of accommodative activity, although hyperopic defocus would result in an even more constant contrast signal. The results of lens-induced defocus experiments are explained in the same manner (section 4.4).

In monochromatic light the eye uses the non-chromatic cue, i.e. changes in contrast, to direct growth and thus emmetropization responses are seen. However, as fine tuning information due to longitudinal chromatic aberration is absent, differences in focus due to the power of the eye varying with wavelength are not compensated for (section 5.2). When longitudinal chromatic aberration is present emmetropization to low levels of spectacle induced defocus occur (section 3.3). The variation in contrast of different wavelengths with defocus is independent of the eyes depth-of-focus and thus very small changes in defocus can be compensated for. Emmetropization still occurs when variations in object contrast are removed as the longitudinal chromatic aberration cue is present. As longitudinal chromatic aberration is most effective at guiding accommodation at intermediate spatial frequencies, this explains the dependence of emmetropization on the spatial frequency of the image.

A visual feed-back system using longitudinal chromatic aberration and contrast as defocus cues is suggest (Fig. 7.2). The defocus is detected by the refractive error detection system and the appropriate growth signal generated. After some indeterminate period a defocus signal is again generated, presumably smaller, and the system repeats itself until emmetropia is obtained.

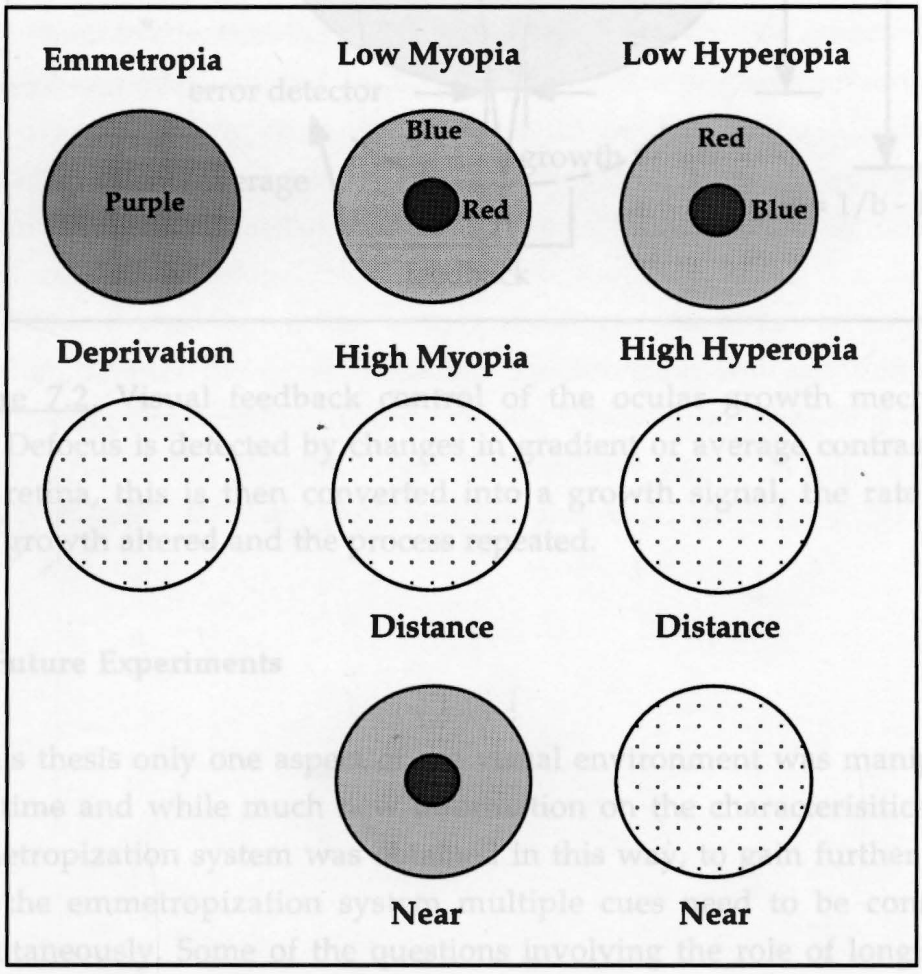


Figure 7.1. Retinal blur circles for the longitudinal chromatic aberration and contrast error detector model.

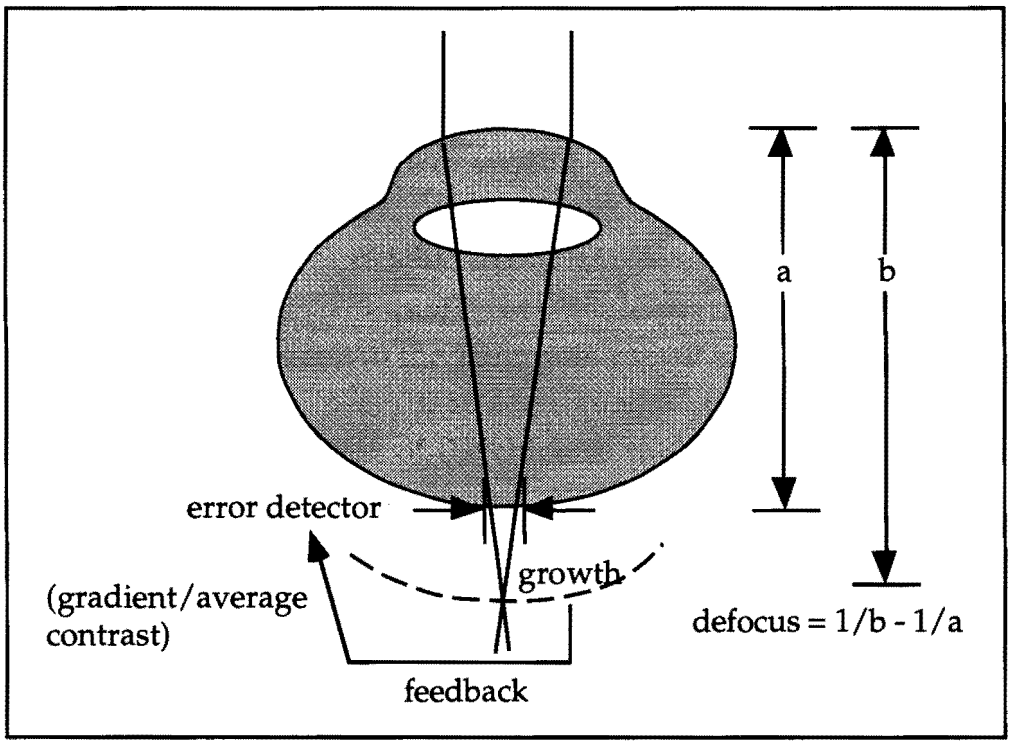


Figure 7.2. Visual feedback control of the ocular growth mechanism. Defocus is detected by changes in gradient or average contrast at the retina, this is then converted into a growth signal, the rate of eye growth altered and the process repeated.

7.3. Future Experiments

In this thesis only one aspect of the visual environment was manipulated at a time and while much new information on the characterisitics of the emmetropization system was obtained in this way, to gain further insight into the emmetropization system multiple cues need to be considered simultaneously. Some of the questions involving the role of longitudinal chromatic aberration in emetropization could be answered by repeating the spatial-frequency experiment under monochromatic light conditions. If the spatial- frequency-dependence observed under white light is still observed under monochromatic light this would indicate that the dependence was not a result of longitudinal chromatic aberration and provide greater support for other non-chromatic defocus cues being involved. Conversely, if the spatial-frequency-dependence of emmetropization is lost under monochromatic light conditions this would argue in favour of a role for chromatic aberration Also to give

further meaning to the contrast and spatial frequency experiments, determination of the contrast sensitivity function of the chick is required.

One of the key findings of recent studies in this field of eye growth control was the fact that eye growth appears to be locally controlled in the chick (Wallman, Gottlieb *et al.*, 1989). The studies reported here do not address whether the restricted contrast and spatial frequency environments provide adequate or inadequate information for emmetropization if feedback to the brain was prevented.

Similarly while it appears that accommodation is not fundamental to the emmetropization system of the chick, in the studies reported in this thesis chicks were reared in a normal visual environment with many potential alternative visual cues. It would be interesting to repeat the spectacle lens experiment using restricted visual environments to determine if accommodative input becomes important under conditions of reduced information.

While it was shown that a myopic defocus signal can be detected in less than 20 min the exact threshold for detection was not determined. Determining the threshold could provide information regarding the relay of the signal to the choroid, where the earliest changes in eye growth are seen, (Wallman, Xu *et al.*, 1992) and the pathway involved.

APPENDIX I

METHODS IN DEPTH

AI.1. Methods of Myopia Production

AI.1.1. Lid Suture

Technique

Lid suture was performed under a M650 Leitz surgical microscope. Tissue, 1/2 mm to 1 mm thick, was trimmed off the upper and lower eyelid margins which were subsequently sutured together with silk suture thread (Ethicon, 9/0); six to eight sutures were inserted. An antibiotic (Sofradex, Roussel pharmaceuticals; contains: soframycin, gramicidin, dexamethasone sodium metasulphobenzoate) was applied prophylactically and the wound sealed with superglue. Chicks were anaesthetized with 2% fluothane (halothane) in oxygen (1.0 litre/min) during the procedure. The fused lids healed to produce a thin translucent membrane over the eye which prevented high quality form vision. The nictitating membrane appeared to function normally beneath the fused lid.

Effect on retinal image

Lid fusion, in monkey and cat, has been shown to have four major effects on the retinal image, reduced illumination, decreased image contrast at all spatial frequencies, loss of high spatial frequencies and altered spectral composition of the image (Crawford and Marc, 1976; Loop and Sherman, 1977). Recently it has been reported that for the chick lid suture similarly reduces light levels, with extensive attenuation of light below 460 nm and a progressive increase in transmission, with increasing wavelength, to a maximum of 40% (Wildsoet, 1992).

AI.1.2. Occluders

Technique

As an alternative to lid suture, translucent white plastic domed occluders were applied to the eyes of chicks. Occluders were glued directly to the feathers surrounding the eye except in cases where the occluder had to be removed and replaced, e.g. section 3.1. In those cases, the occluders were attached to one half of a velcro ring support and the other half was superglued to the feathers surrounding the eye of the chick (Plate AI.1).

To grossly determine the effects of the occluders on the retinal image, the intensity of light from a fibre optic light source was measured using a light meter with and without an occluder in the light path (n = 6). This showed that the occluders reduced the light intensity reaching the eye to approximately one tenth of that without an occluder. The occluders also prevent detailed form vision when held in front of a human observer's eye.

AI.2. Lenses

AI.2.1. Spectacle lenses

The spectacle lenses (sections 3.3, 3.4, 4.1, 4.2) were 12 mm diameter modified human PMMA contact lenses (Plate AI.1). The lenses had large optic zones (10.5 mm to 11.5 mm) and 8.0 mm back optic radi and allowed panoramic vision. The lenses were applied to the chick eyes by means of a velcro ring support, one side being glued to the feathers surrounding the eye of the chick and the other to the lens; this enabled the lenses to be removed and replaced for cleaning.

Plate AI.1

(overleaf)

Plate AI.1. Examples of the occluders and spectacles lenses used. In **A**. a monocularly occluded chick is sown and in **B**. a spectacle lens wearing chick. Occluders and lenses could be easily removed due to the use of velcro.


A

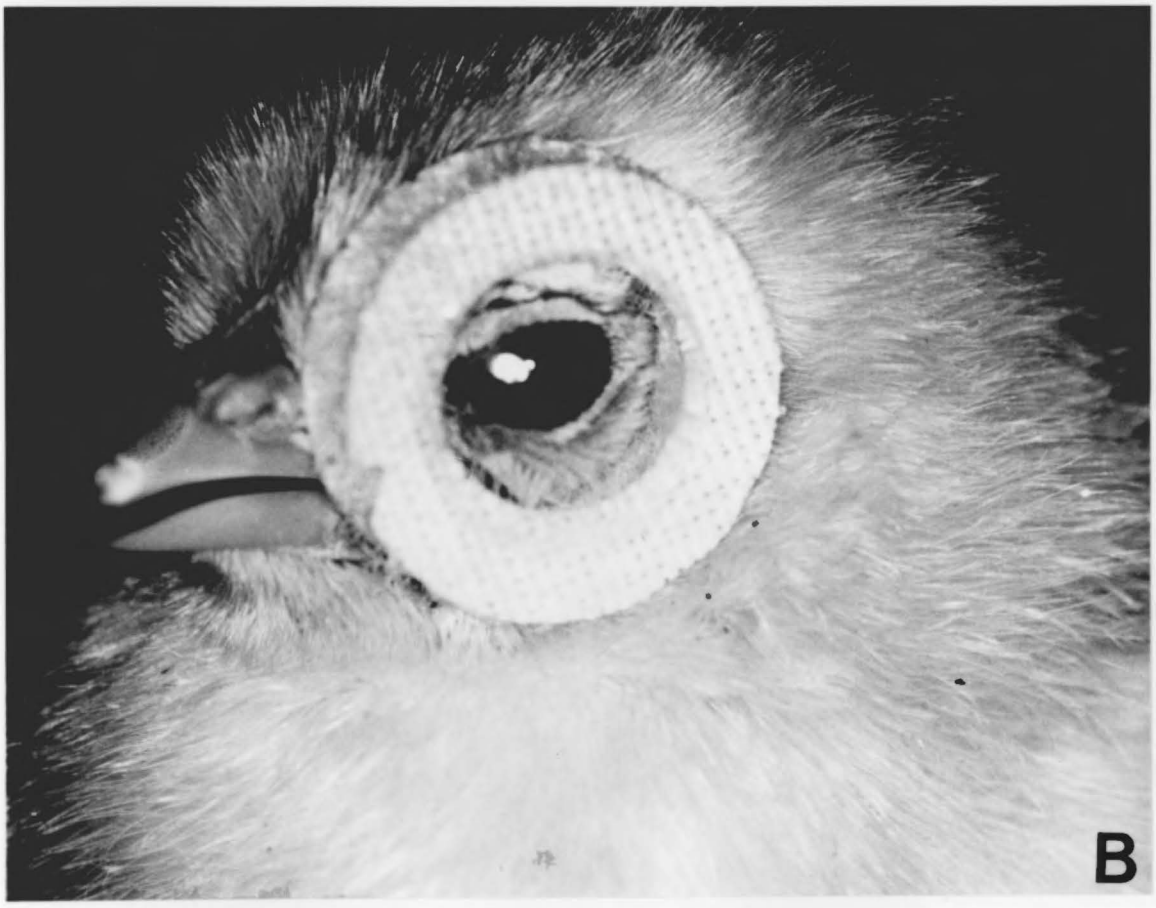

B

Plate AI.2

(overleaf)

Plate AI.2. The hard contact lens on the chick eye. In **A**. the fluorescein pattern indicates a large negative tear layer as typical for a "flat lens fit", and in **B**. the bright vertical line (arrowed) is the edge of the nictitating membrane underneath the lens.


A


B

AI.2.2. Hard contact lenses

The hard contact lenses (HCL; section 4.2) were made of PMMA with back optic radi of 3.5 mm, lens diameter of 6.0 mm and back vertex power of 0 D. As a visual aid in assessing lens centration and location, the lenses had a metallic gold ring painted around the edge of the lens; approximately 0.5 mm thick. The lenses had to be fitted "large and flat" to obtain adequate stability; the nictitating membrane of the chick passed underneath the lens in this case (Plate AI.2). This lens design was adequate for chicks in the age range 1 to 14 days. The lens parameters were not appropriate for the larger corneas of older chicks outside this range. Monocular hard contact lens wear was well tolerated by the chicks; while chicks occasionally scratched at the eye wearing the lens, this occurred no more frequently then for the spectacle lenses. Corneal clouding or eye irritation, i.e. redness, was not seen for the duration of the study.

AI.3. Ocular Measurements

AI.3.1. Retinoscopy

Measurement protocol

Static retinoscopy was used to determine refractive errors. Chickens were anaesthetized with 1.5% to 2.5% fluothane (halothane) in oxygen (1.0 to 1.5 litre/min), the dose varying with the size and age of the chicken. Relatively deep anaesthesia was used to obtain good pupil dilation and to minimize accommodative activity. Custom-made lid retractors were used to hold the lids open during measurements. A Heine streak retinoscope and standard trial ophthalmic lenses were used. Retinoscopy was performed in a darkened room to aid visualization of the retinoscopic reflex. Measurements were made along the two principal meridians of the eye, the recorded result being the most hyperopic value observed in each meridian; relatively more myopic values were assumed to be due to accommodation. These values for the two meridians were averaged to give the spherical equivalent refraction of each eye; their difference gave the amount of astigmatism.

Source of errors

Noncycloplegic refractions were performed due to the difficulty in producing cycloplegia in birds and to minimize disruption to vision during on-going experiments involving periodic measurement. Wallman and Adams (1987) found that there was no significant difference between cycloplegic and noncyclopegic refractions for the chicken.

Also, refractions presented in this thesis have not been corrected for the small eye retinoscopic artifact. The calculated artifact of retinoscopy: artifact = $(n_V \times T_{ret}) / [0.85 \times \text{axial length} \times (\text{axial length} - T_{ret})]$ where n_V is the refractive index of the vitreous and T_{ret} the thickness of the retina, is greater for small eyes (Glickstein and Millodot, 1970), particularly those of less than 10 mm in axial length (Wallman and Adams, 1987). This artifact is postulated to occur as a result of the retinoscopic beam coming from the retinal-vitreous boundary rather than from the photoreceptor layer of the retina (Millodot and Sivak, 1978). The magnitude of this artifact has been estimated to account for approximately 4 D, 3 D and 2 D of the measured hyperopia of normal chicks at 2, 4 and 8 weeks respectively (Wallman and Adams, 1987). This artifact was not taken into account in measurements reported in this thesis because: i) accurate in vivo retinal thickness measurements were not possible, and ii) the significance of this artifact is still subject to debate (Hughes, 1979; Green *et al.*, 1980; Schaeffel and Howland, 1988).

AI.3.2. Chromoretinoscopy

Equipment

Chromoretinoscopy (Bobier and Sivak, 1980) was used to determine the chromatic aberration of the chick eye, a schematic representation of the chromoretinoscope is shown in Figure AI.1. The use of interference filters to obtain monochromatic light had the effect of greatly decreasing the light intensity, for this reason an American Optical retinoscope was modified by replacing its bulb with an optic fibre and Xenon lamp (Oriel, 6255, 150W Xe) combination. The retinoscope was modified in this way due to the need for a high intensity light source; the Xenon lamp emits relatively high intensity light across the entire visible spectrum (Fig. AI.2). In the light path, before the fibre optic, was placed an infra-red heat filter

to absorb heat, and then an interference filter to restrict the wavelength of the light as indicated. The range of interference filters (656±10 nm, 623±10 nm, 550±10 nm, 510±10 nm, 470±10 nm, 450±10 nm, or 420±10 nm) was selected so as to correspond to the wavelengths used in section 5.2.

Measurement protocol

Chicks were anaesthetized using a 2:1 mixture of ketamine (10 mg/ml) and Rhompun (20 mg/ml) using a dose of 0.5 ml/kg and given intramuscularly. As greater accommodative fluctuations occur with this anaesthesia and also as small accommodative changes would have a large effect on the measured chromatic aberration (Charman and Tucker, 1978), cycloplegia was used. A 4:1 mixture of vecuronium bromide (Norcuron, 5 mg/ml) and benzalkonium chloride (Zephiran, 0.13%) was used as a cycloplegic agent; three drops were applied, one every 5 mins. A pen torch was used to check pupil function. After 30 to 40 minutes the eye became widely dilated and unresponsive to light, it was then assumed that cycloplegia had been obtained and chromoretinoscopy was performed. Refractions were measured as in conventional retinoscopy with the order of interference filter randomized.

Source of errors

Chromoretinoscopy is an accepted technique for the measurement of chromatic aberration in animals. Bobier and Sivak (1980) have reported that measurements have a sensitivity to within ±0.10 D. A likely source of error is that associated with the chromatic aberration of trial lenses used to obtain neutralization. The focal length of a lens depends on the wavelength of light; due to the higher refractive index for blue the focal length of a lens will be shorter for blue light than for red light, regardless of whether it is a positive or a negative lens. The error associated with this effect was calculated using the formula (Atchison *et al.*, 1993): $CE\lambda = CEmeas\lambda \times (n\lambda - 1) + \Delta F \times (n\lambda - nref) / (nref - 1)$ Over the range in power of ophthalmic lenses used to measure chromatic aberration, this error source is minimal, leading to longitudinal chromatic aberration being over-estimated by only 0.1 D. The values reported for longitudinal chromatic aberration in the chick have thus not been corrected for this error.

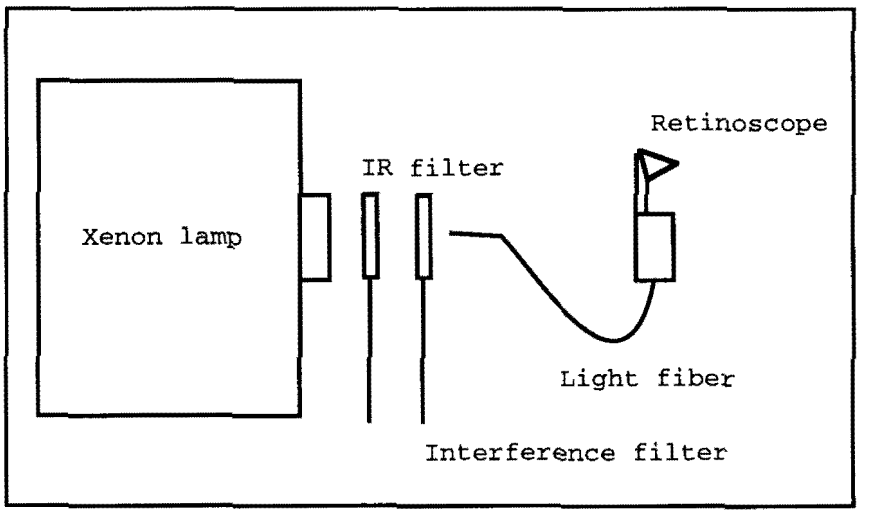


Figure AI.1. Schematic illustration of the chromoretinoscope used to determine the chromatic aberration of the chick eye. A Xenon lamp acted as the light source, replacing the standard bulb of the AO retinoscope and was linked to it by an optical fiber. An infra-red (IR) filter was used to absorb heat, the interference filter limited the wavelength of light.

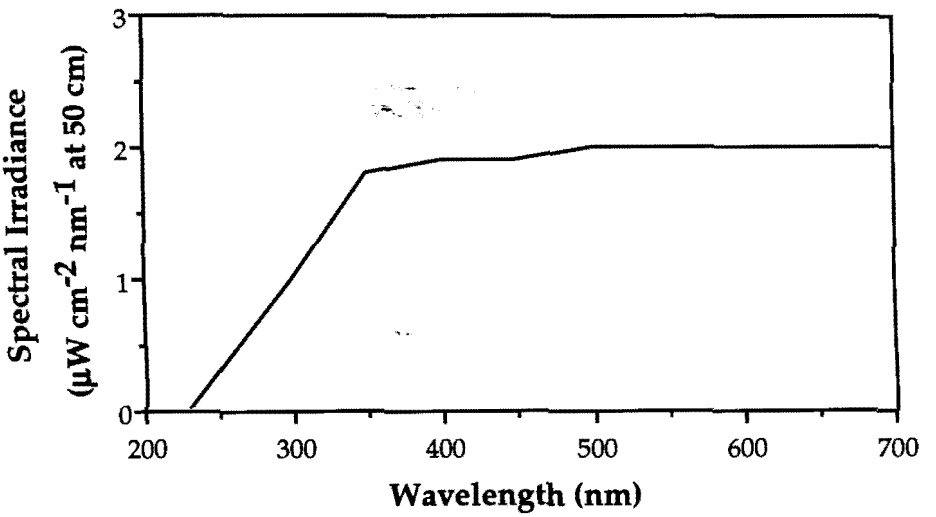


Figure AI.2. Spectral irradiance of the Xenon lamp. The lamp emits at uniformly high intensity across the visible spectrum (adapted from manufacturer specifications).

Calculated longitudinal chromatic aberration

Estimates of longitudinal chromatic aberration, between 420 nm and 656 nm, based on the axial length of the chick eye at different ages were also made. For these calculations it was assumed that the eye was water filled and that the axial length approximated the posterior nodal distance. Estimates of the refractive index of water for different wavelengths were made using Houstoun's dispersion formula: $n^2 = -(1.38 \times 10^6)\,\lambda^2 + 1.7642 + (6.12 \times 10^{-11})\,\lambda^{-2} + (1.41 \times 10^{-20})\,\lambda^{-4}$, where λ is in cm.

This gave values of 1.3420, 1.3348 and 1.3316 for 420 nm, 550 nm and 656 nm respectively. The power of a standard eye was calculated using $P_{st} = n_{ref}/AL_{st}$ for different ages (Atchison *et al.*, 1993); n_{ref} is the refractive index of water at the reference wavelength and AL_{st} the axial length of the standard eye measured using A-scan ultrasound. As measured refractive errors using 550 nm light were most similar to those obtained using white light, 550 nm was used as the reference wavelength. The chromatic differences of focus associated with 420 nm and 656 nm were calculated using $CE\lambda = P_{st} \times (n_{ref} - n\lambda)/\, n_{ref}(n_{ref} - 1)$ and the values added to give an estimate of longitudinal chromatic aberration. Due to the approximations used here calculated values should under-estimate the actual longitudinal chromatic aberration of the chick eye.

AI.3.3. A-scan Ultrasonography

Equipment

A-scan ultrasonography was used to measure the axial ocular dimensions on anaesthetized chickens (Fig. AI.3; Plate AI.3). Chickens were anaesthetized using halothane and a lid retractor used to hold open the lids during measurements. The apparatus included a focussed transducer (Panametric, 7.5 MHz) attached to a pulser-receiver (Panametric), with the output going to an oscilloscope and then computer for the majority of measurements. The transducer was mounted on an adjustable metal arm which could be tilted to any required angle to facilitate alignment. A plastic cone was attached to the transducer so as to achieve appropriate "stand-off"; the cone was filled with water, covered with parafilm and then coated with Lacrilube (Allergan; contains: soft white paraffin, liquid paraffin, nonionic wool fat derivative, chlorbutol) which provided the ultrasound contact between the cone and the chick's cornea. The

oscilloscope was used to view and store the returning signal which was subsequently down loaded to a computer for on-line analysis.

Measurement protocol

Anterior chamber depth, axial lens thickness and vitreous chamber depth were measured by this technique. The alignment of the ultrasound probe was varied so as to maximize the echoes produced by reflection at both the anterior and posterior surfaces of the lens and hence alignment with the optic axis of the eye (Gollende *et al.*, 1979; Wallman and Adams, 1987). The time separation between reflections from the anterior corneal surface, anterior and posterior lens surfaces and retina were measured either directly off the oscilloscope screen or with the aid of a computer program. The time measurements were then converted to linear distances using estimates of the velocity of ultrasound in the relevant ocular media for the chicken eye; values used were 1.534 mm/μsec for both the aqueous and vitreous and 1.6078 mm/μsec for the lens as determined by Wallman and Adams (1987). Three measurements were recorded for each eye, the transducer probe being realigned between measures. The averages of these readings were used in later analysis.

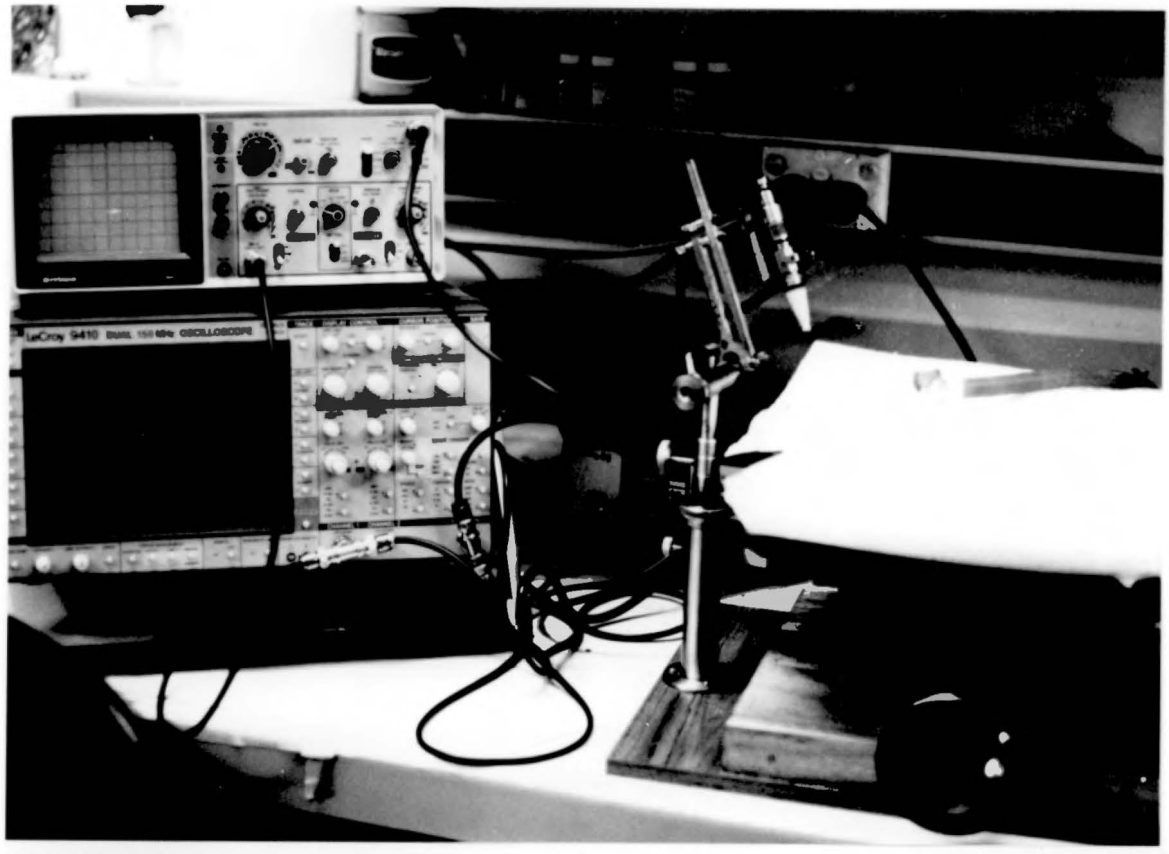
Source of errors

The main source of error with this technique is inaccurate alignment. Repeated measurements of anterior chamber depth, lens thickness and vitreous chamber depth gave standard deviations of less than 0.05 mm which is similar to that previously reported (Troilo *et al.*, 1987). In all cases, the anterior chamber depth measurement includes the thickness of the cornea. This strategy was adopted because the ultrasound probe did not allow resolution of anterior and posterior corneal reflections and hence measurement of corneal thickness.

Plate AI.3

(overleaf)

Plate AI.3. The eye growth monitoring equipment. In **A**. the A-scan ultrasound used for monitoring the ocular dimensions is shown, and in **B**. the photokeratometer and infrared-photoretinoscope camera attachments that are used for corneal power measurements and accommodative facility respectively are depicted.



B

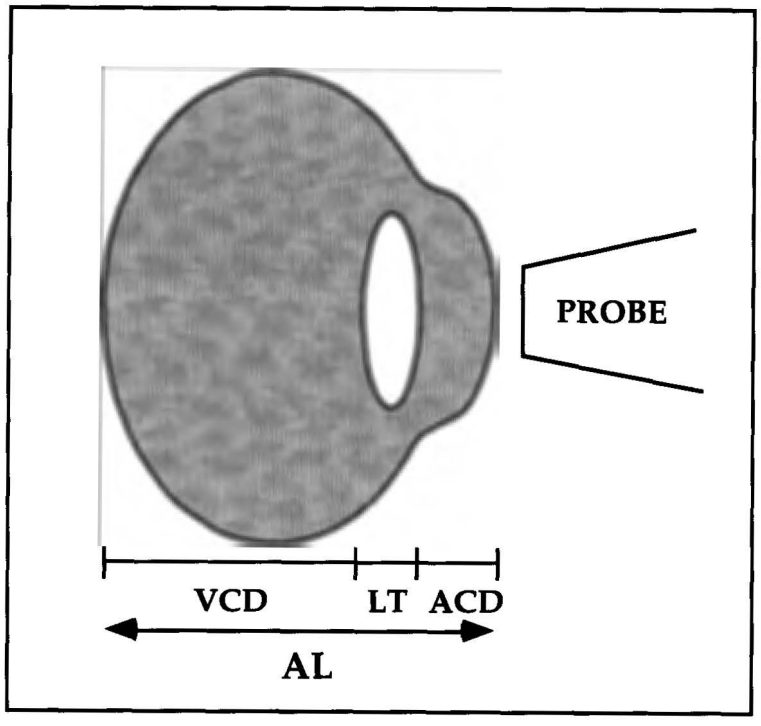


Figure AI.3. A-scan ultrasound measurement of the ocular components of the chick eye; anterior chamber depth (ACD), axial lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL) were measured with this technique.

AI.3.4. Photokeratoscopy

Equipment

The equipment used for video-infra-red-photokeratometry is shown in Plate AI.3. The infra-red (IR) photokeratometer consisted of a ring of 8 infra-red light emitting diodes (LEDs), mounted in movable plastic spheres to aid angular alignment, and inserted around the margin of a hexagonal plastic support base. The LEDs were powered by a 6 volt power supply. The distance between adjacent LEDs was 13 cm and between opposite LEDs was 33 cm, i.e. the radius of circle on which the LEDs were placed was 16.5 cm. The support base was located between the C mount adaptor and an IR-sensitive Panasonic WV BL-200 video camera which was used to capture images reflected from chick corneas. The video camera fitted with a Micro Nikkor 105 mm lens was linked to a Sony Video 8 recorder. Video tape recordings were analyzed using a frame

grabber and image processing software (Image 1.30) on a Macintosh computer.

Measurement protocol

Chicks were anaesthetized using a 2:1 mixture of ketamine (10 mg/ml) and Rhompun (20 mg/ml) using a dose of 0.5 ml/kg and given intramuscularly. A lid retractor was used to hold open the lids during measurements. Chicks were positioned so that the IR LED reflections from the cornea were in focus and centreed about the pupil. The image was then captured onto video tape. As a calibration check, reflections from a ball bearing, 11.1 mm in diameter, were captured at the commencement and completion of each session.

Video images were analyzed at a later date. Video frames selected on the basis of image clarity and centration were "grabbed" and analyzed by computer; the linear distance between IR reflections, along the horizontal and vertical axis, were measured in pixels. Three such frames were analyzed for each eye and the results averaged. The measured pixel distances, which represent image separations for diametrically opposite IR LEDs, were converted to corneal power in diopters using a computer program developed by Howland (Fig. AI.4); screen distortions were accounted for (Howland and Sayles, 1985).

Source of errors

It has been stated that photokeratometry is the most sensitive method to measure corneal curvature in chicks (Schaeffel and Howland, 1987); due to their steep corneas, standard keratometry is not appropriate. However, photokeratometry is not a method devoid of measurement errors. Schaeffel and Howland (1987) found that the measured corneal power was most affected by variation in the distance of the chick eye to the camera (C) and the distance of the chick eye to the photokeratometer (D). They found that a displacement of the eye of 1 mm along the optic axis resulted in an error of about 1 D, due to the depth of focus of the video camera system (also approximately 1 mm); the expected accuracy of a single measurement is ±1 D. The reliability was improved for repeated measures.

Another source of error was linked to corneal asphericity, i.e. the increase of corneal radius of curvature with increasing distance from the

pupil centre (Schaeffel and Howland, 1987). With the apparatus used in the present work, the corneal region assessed was only slightly peripheral and hence the measured corneal power expected to be at worst only slightly less than the true corneal power. This was felt to be a reasonable compromise, as measurements close to the centre of the pupil are more sensitive to measurement errors due to the decreased separation of the reflections.

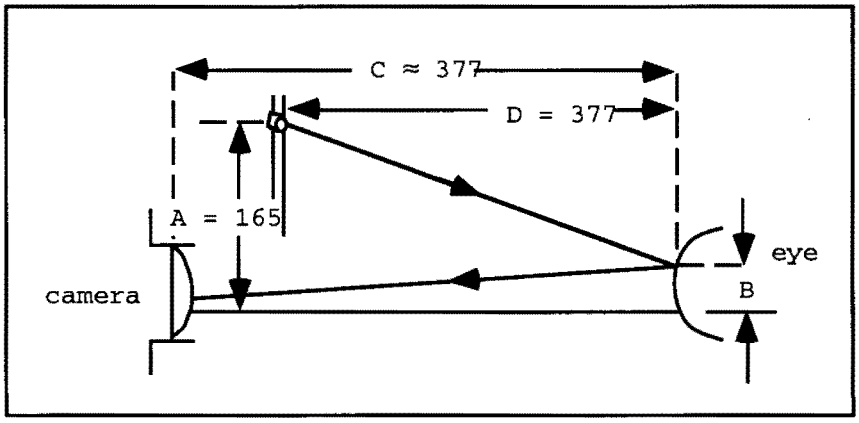


Figure AI.4. Schematic representation of the photokeratoscope. Corneal powers were calculated using the equation R = B/ sin 0.5 {arctan[(A-B)/(D-0.5R)]} - B/ sin 0.5 {arctan[B/(C-0.5R)]}. Where R is the corneal radius of curvature, B is the half distance between opposite reflections, A the distance of the IR LEDs from the optic axis (165 mm), D the distance from the IR LEDs to the plane of reflection in the corneal surface (377 mm) and C the distance from the plane of reflection in the corneal surface to the principal plane of the camera (≈377 mm).

Accuracy

The reliability of the photokeratometer was checked using a series of steel balls of known curvature, with radi ranging from approximately 3.0 to 7.0 mm. A comparison of the radius of curvature measured by keratometry to the actual radius of curvature as measured by calipers was made (Fig. AI.4). Measured values were highly correlated to actual values and were not statistically different from each other (r = 0.999, $P < 0.001$). There was a slight tendency to underestimate corneal curvature for large ball bearings

however this effect was insignificant and did not affect the range of curvatures usually encountered in the chick eye, i.e. approximately 2.5 mm for very young chicks to approximately 4.5 mm for 8 week-old chicks.

The accuracy of this technique was also assessed using repeated measurements (n = 6) of the steel balls. The average error was less than 0.25 D (i.e. SD/√n); it should however be mentioned, that this is a measure of best performance and that errors associated with actual corneal curvature measurements are likely to be larger due to: i) poorer image quality than with steel balls, especially for highly myopic chicks with very steep corneas, ii) misalignment errors due to the aspheric nature of the chick cornea, iii) residual accommodative activity (although more practical for keratometry measurements, ketamine/Rhompun anaesthesia seems poorer than halothane at controlling accommodation; author observation), and iv) the lid retractors may alter corneal curvature if they exert pressure on the eye.

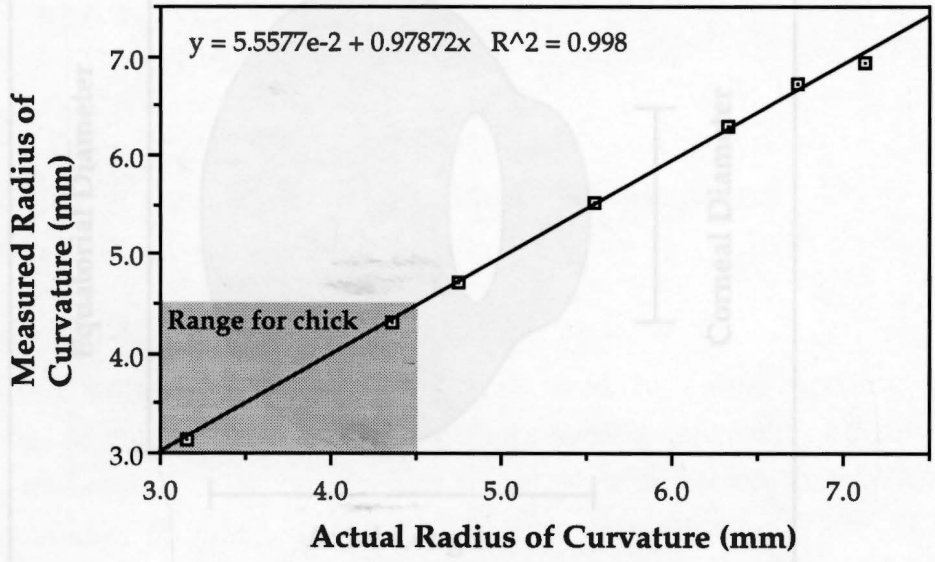


Figure AI.4. Measured compared with actual radius of curvature of ball bearings (r = 0.999, P < 0.001). The shaded zone represents the range of curvatures normally seen for the chick.

AI.3.5. Caliper measurements

Measurement protocol

External dimensions of enucleated eyes were measured using digital calipers; axial length, horizontal and vertical equatorial and corneal diameters were measured. Animals were sacrificed with an overdose of sodium pentobarbitone (Lethabarb, 60 mg/kg I.P.). The eyes were then dissected free of the surrounding muscle, optic nerve and loose connective tissue (Fig. AI.5). The horizontal and vertical equatorial and corneal diameters were averaged respectively to give average equatorial and corneal diameters. For each parameter, three measurements were averaged; all standard deviations were less then 0.05 mm.

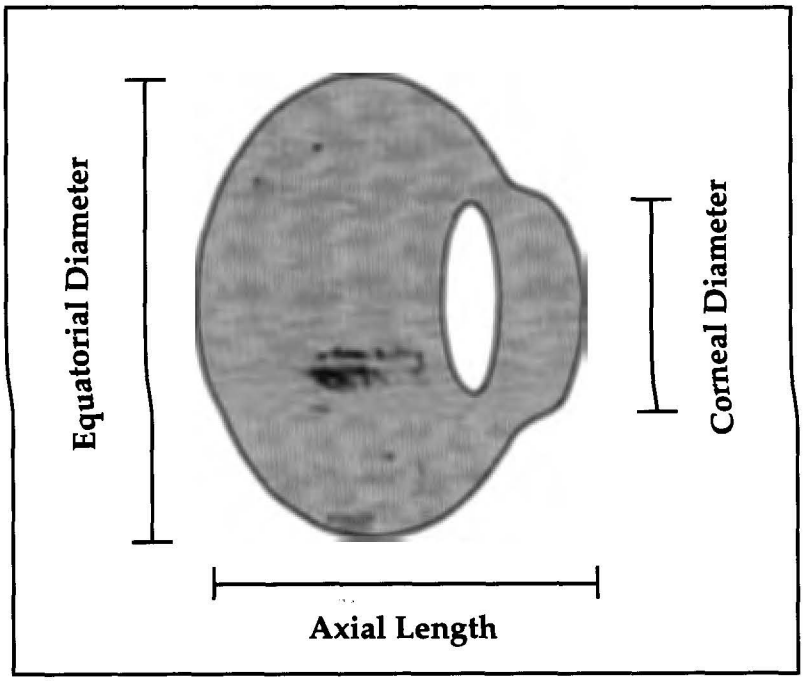


Figure AI.5. External measurements of the eye made with calipers; corneal diameter, equatorial diameter and axial length measurements were made with this technique.

AI.4. Accommodation

AI.4.1. Ciliary Nerve Section

Technique

Chickens were anaesthetized with 2% fluothane (halothane) in oxygen (1.0 litre/min) for ciliary nerve section (CNS) surgery. The operation was performed under a M650 Leitz surgical microscope. The posterior orbit was accessed via a small incision, 2 to 3 mm, made through the lower lid and the extraocular muscle cone split apart with forceps to expose the nerve (Plate AI.4). The nerve was then hooked and cut on the bulbar side of the ciliary ganglion. Two sutures (Ethicon, chromic gut, 6/0) were used to close the small incision, a prophylactic antibiotic (sofradex) applied and the wound sealed with superglue.

The success of the surgery was verified by examining: i) pupil reactions; a widely dilated, unresponsive pupil was confirmed, and ii) infra-red video-photoretinoscopy (Schaeffel *et al.*, 1987); no accommodative activity could be elicited.

AI.4.2. Infra-Red Video-Photoretinoscopy

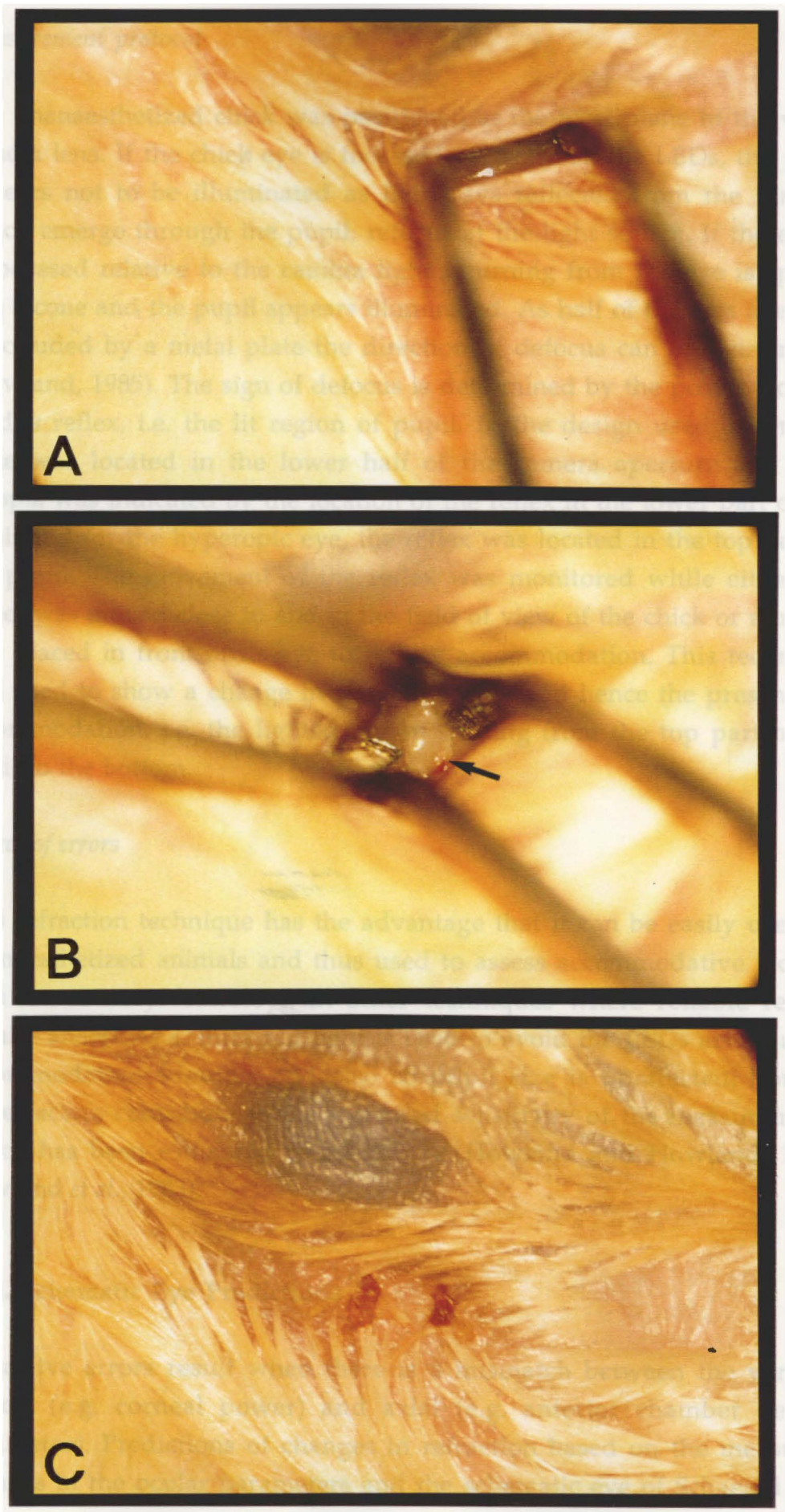
Equipment

Infra-red video-photoretinoscopy was used to assess accommodative function in chicks. The IR LED photoretinoscope consisted of 5 rows of IR LEDs embedded in a semicircular metal plate fitted on to the front of a Micro Nikkor 50 mm lens via a 52-58 mm stepping ring (Plate AI.3). The number of LEDs per row decreased by 1 LED per row, from 2 LEDs to 6 LEDs. The rows were positioned at different eccentricities (2 mm, 6.5 mm, 11 mm, 15.5 mm, 20 mm) from the optical axis of the lens which was attached to an IR-sensitive video camera (Panasonic WV BL-200). The controls of the photoretinoscope allowed alternative rows to be lit in sequence or alternately, individual rows of LEDs could be selected. The LEDs were powered by a 9 volt power supply. The camera was linked to a Sony Video 8 recorder. Analysis of the video tape was carried out using a frame grabber and image processing software, Image 1.30, installed on a Macintosh computer.

Plate AI.4

(overleaf)

Plate AI.4. Ciliary nerve section surgery: **A**. the tissue was gently prised apart with forceps; **B**. the ciliary ganglion (arrowed) was exposed, hooked and cut; **C**. the wound was sutured closed and sealed with superglue.


A
B
C

Measurement protocol

The unanaesthetized chick was positioned at the focal plane of the video camera lens. If the chick eye is focussed perfectly on the LEDs, the pupil appears not to be illuminated as light rays reflected from the fundus, which emerge through the pupil, refocus at the light source. If the eye is defocussed relative to the camera light returning from the eye is spread into a cone and the pupil appears illuminated. As half of the lens aperture is occluded by a metal plate the direction of defocus can be determined (Howland, 1985). The sign of defocus is determined by the position of the fundus reflex, i.e. the lit region of pupil. In the design used, the metal plate was located in the lower half of the camera aperture and thus myopia was indicated by the location of the reflex in the lower part of the pupil and for the hyperopic eye, the reflex was located in the top part of the pupil. The movement of the reflex was monitored while either, an object was moved close to and in the field of view of the chick or a minus lens placed in front of its eye to induce accommodation. This technique was used to show a change in refractive state and hence the presence of accommodation, i.e. the lighted region shifting from the top part of the pupil to the bottom.

Source of errors

This refraction technique has the advantage that it can be easily used on unanaesthetized animals and thus used to assess accommodative facility. This is not very feasible with other techniques where reliable results require anaesthesia. Infra-red light is used to avoid the LEDs acting as an accommodative stimulus and also so pupil size is unaffected; both of these factors contribute to the increased sensitivity of the measurements which has been estimated to be ±0.5 D (Howland and Howland, 1974; Howland *et al.*, 1983).

AI.5. Schematic Eye Predictions

Refractive errors result when there is a mismatch between the various optical (e.g. corneal power) and axial (e.g. anterior chamber depth) parameters. Predictions of changes in refraction based on the measured changes in the ocular parameters and the schematic eye of Schaeffel and

Howland (1988a; Fig. AI.6) were made for comparison to actual measured changes in refraction. It should be noted that this eye model is for a 30 day-old chick and thus predictions may be inaccurate for young chicks.

The formula used for calculation were (Wildsoet, 1992):

$$\Delta RE_{Total} = \Delta RE_{ACD} + \Delta RE_{VCD}$$
$$\Delta RE_{ACD} = -5.39 \times \Delta ACD \times 5.39$$
$$\Delta RE_{VCD} = -15.817 \times \Delta VCD$$

Abbreviations: ΔRE = difference in refractive error of treated and normal eyes; ΔRE_{ACD} = predicted difference in refractive error due to changes in anterior chamber depth; ΔRE_{VCD} = predicted difference in refractive error due to changes in vitreous chamber depth; predicted ΔRE = predicted change in refractive error based on sum of ΔRE_{ACD} and ΔRE_{VCD}.

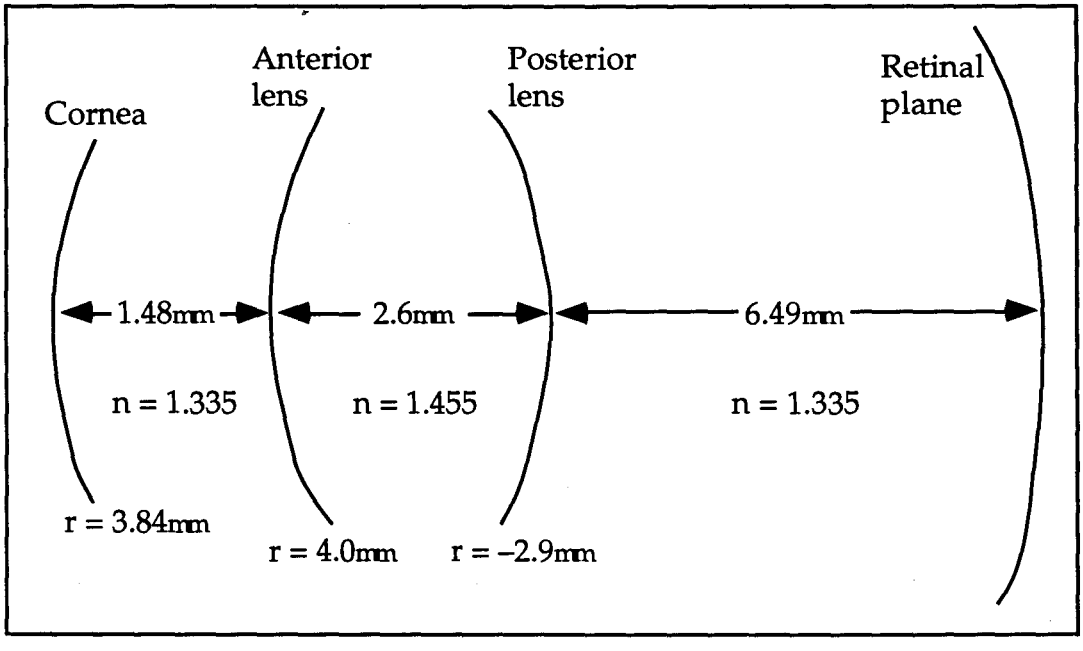


Figure AI.6. Schematic eye based on data of Schaeffel and Howland (1988a) used to determine predicted changes in refraction (Not to scale).

In addition to the above, differences in corneal power were also taken into account in the predictions used in this thesis. In contrast, the predictions did not take into account changes in lens thickness or lens curvature.

AI.6. Depth-of-Focus

The depth-of-focus of the chick eye was estimated by using the equation (Green *et al.*, 1980): $\Delta D = 17.45\ \phi / p$, where ϕ is the minimum resolvable blur circle diameter in degrees and p the pupil size in mm. The depth-of-focus was calculated using both the anatomical acuity, estimated from Ehrlich (1981), and the behavioural acuity (Over and Moore, 1981) for pupil sizes varying from 0.8 mm to 4.6 mm. The anatomical acuity was that estimated for a 30 day-old chick, acuity may be poorer in younger chicks and the depth of focus commensurably greater. Small eyes with low visual acuity have large depth-of-focus (Green *et al.*, 1980). Focal length is not used in this equation as changes in length produce equivalent changes in blur circle diameter and retinal image size. It should also be noted that this equation is an approximation only and diffraction effects were not considered.

AI.7. Statistics

The choice of statistical test was based on information in Siegel (1956) or on advice given. Mostly, nonparametric tests have been used due to their usefulness with small sample sizes and as the data are neither normally distributed nor do experimental groups possess equal variance. The size of samples was determined by considerations of both the number of animals needed for statistical validity and ethical limits. Typically six chicks are assigned to each treatment group in research involving the chick as a model for eye growth. Experiments reported in this thesis use animal numbers slightly greater than six; the exception being where the results of some animals have had to be rejected and in those cases the minimum sample size used is six .

AI.6.1. Wilcoxon matched-pairs signed-ranks test (WSRT)

The Wilcoxon matched-pairs signed-ranks test has been used when comparing the data from two related samples. It utilizes information about both the direction and magnitude of the differences within pairs. The rejection region was set at $\alpha \leq 0.05$ for both the one- and two-tailed versions of the test. Where the direction of the difference could be predicted, a one-tailed test was used, in other cases, the two-tailed test was used. This test has been used for within chick comparisons of treated and normal eyes.

AI.6.2. Mann-Whitney U-test (MWUT)

The Mann-Whitney U-test is used when comparing the data from two independent samples. It utilizes information about both the direction and magnitude of the differences between groups. The rejection region was set at $\alpha \leq 0.05$ for both the one- and two-tailed versions of the test. Again where the direction of the difference could be predicted, the one-tailed test was used and otherwise the two-tailed test was used. This test has been used when comparing different treatment effects and as an alternative to the unpaired student *t* test; in this case treatment effects were usually expressed as differences between treated and control eyes of the same chick.

AI.6.3. Linear regression

Linear regression analysis was used to determine the relationship between different ocular parameters. Correlation coefficients were determined and the rejection region set at $\alpha \leq 0.05$.

AI.8. Ethics

All experiments were conducted in accordance with the "Australian code of practice for the care and use of animals for scientific purposes" of the NHMRC. Chick numbers were kept to the minimum possible for statistical analysis.

Table AII.2.1.1. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 7 both groups).

Ocular parameter		Week 1		Week 2		Week 3	
		WL	B	WL	B	WL	B
Refraction (D)	Treated eye	–	–	−24.5±2.5***	−18.4±2.8***	−17.0±3.9***	−4.6±3.9**
	Normal eye	+3.1±0.4	+1.1±0.6	+0.8±0.7	+1.2±2.3	+1.2±0.4	+2.1±2.1
Corneal power	Treated eye	–	–	109.4±3.9*	108.0±2.9	101.4±2.5	103.3±2.9
(D)	Normal eye	115.3±2.2	117.0±3.1	105.6±1.3	109.7±1.7	100.3±2.9	99.0±2.0
Anterior chamber	Treated eye	1.37±0.04**	1.18±0.08	1.96±0.07***	1.86±0.12***	2.33±0.12***	2.07±0.11***
depth (mm)	Normal eye	1.27±0.02	1.28±0.05	1.45±0.03	1.51±0.05	1.61±0.04	1.70±0.04
Axial lens	Treated eye	2.19±0.03	1.99±0.02	2.38±0.03	2.11±0.03	2.51±0.03	2.28±0.04
thickness (mm)	Normal eye	2.18±0.03	1.98±0.02	2.38±0.03	2.13±0.03	2.52±0.03	2.31±0.03
Vitreous chamber	Treated eye	6.02±0.13***	5.98±0.13***	6.99±0.17***	6.85±0.17***	6.51±0.15***	6.33±0.11***
depth (mm)	Normal eye	4.99±0.09	5.23±0.10	5.44±0.09	5.62±0.13	5.82±0.09	6.14±0.09
Axial length (mm)	Treated eye	9.58±0.17***	9.16±0.18***	11.33±0.23***	10.84±0.29***	11.32±0.25***	10.64±0.38**
	Normal eye	8.44±0.11	8.48±0.13	9.27±0.13	9.26±0.15	9.95±0.09	10.14±0.09

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.1.2. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 7 both groups).

Ocular parameter		Week 4		Week 5		Week 6	
		WL	B	WL	B	WL	B
Refraction (D)	Treated eye	–3.1±2.7*	–0.1±1.3	–0.3±1.1	–0.1±1.8	+0.2±0.7	+0.9±1.1
	Normal eye	+1.8±0.4	+0.3±0.5	+1.8±0.4	+0.1±0.7	+1.5±0.7	+0.6±0.7
Corneal power	Treated eye	96.1±4.9	94.4±2.6	90.9±2.2	87.8±2.0	86.4±2.3	81.8±2.1
(D)	Normal eye	92.3±1.4	91.2±0.9	88.7±2.0	86.5±1.7	84.1±0.9	82.1±1.1
Anterior chamber	Treated eye	2.27±0.18*	2.08±0.18*	2.26±0.21*	2.11±0.09	2.20±0.17*	2.25±0.07
depth (mm)	Normal eye	1.76±0.04	1.86±0.05	1.91±0.04	2.07±0.11	2.02±0.05	2.29±0.09
Axial lens	Treated eye	2.70±0.04	2.40±0.04	2.81±0.03	2.55±0.03	2.95±0.04	2.67±0.03
thickness (mm)	Normal eye	2.73±0.03	2.46±0.03	2.86±0.03	2.56±0.04	2.98±0.04	2.71±0.03
Vitreous chamber	Treated eye	6.45±0.24*	6.63±0.15	6.36±0.22	7.01±0.19	6.66±0.21	7.54±0.18
depth (mm)	Normal eye	6.18±0.13	6.72±0.09	6.54±0.17	7.06±0.13	6.83±0.17	7.36±0.19
Axial length (mm)	Treated eye	11.42±0.29*	11.12±0.17	11.44±0.29	11.67±0.21	11.82±0.25	12.46±0.22
	Normal eye	10.67±0.18	11.05±0.13	11.30±0.16	11.69±0.25	11.84±0.21	12.37±0.24

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.1.3. Ocular parameters of treated and normal eyes, occlusion for 2 weeks (mean ± SD, n = 7 both groups).

Ocular parameter		Week 2		Week 3		Week 4		Week 5		Week 6	
		WL	B	WL	B	WL	B	WL	B	WL	B
Refraction (D)	Treated eye	−29.1±3.7***	−19.6±9.3***	−9.0±5.3***	−1.7±3.3**	−0.9±3.3*	+1.3±0.5	+0.6±2.0	+1.1±0.5	+1.3±0.6	+1.0±0.8
	Normal eye	+1.9±0.8	+1.6±1.8	+1.9±0.7	+2.2±0.7	+1.7±0.7	+1.5±0.7	+1.3±0.6	+2.0±0.3	+1.3±0.4	+1.7±0.8
Corneal power	Treated eye	110.0±4.0*	107.7±6.6	94.4±2.6	105.2±2.0	94.4±2.6	99.4±2.4	95.1±2.6	82.8±2.6	89.4±4.3	78.4±3.8
(D)	Normal eye	105.2±3.4	104.8±2.9	91.2±0.9	101.5±1.9	91.2±0.9	96.0±1.8	90.2±1.4	82.3±2.8	85.8±2.0	79.0±3.8
Anterior chamber	Treated eye	2.08±0.25***	1.83±0.28***	2.29±0.18***	1.93±0.21**	2.31±0.40***	1.91±0.09	2.33±0.31**	2.06±0.13	2.34±0.37*	2.26±0.11
depth (mm)	Normal eye	1.45±0.05	1.44±0.04	1.61±0.36	1.63±0.04	1.69±0.16	1.85±0.06	1.87±0.08	2.03±0.09	1.98±0.08	2.20±0.12
Axial lens	Treated eye	2.30±0.04	2.15±0.04	2.45±0.03	2.29±0.05	2.61±0.04	2.45±0.06	2.75±0.05	2.52±0.05	2.87±0.05	2.62±0.04
thickness (mm)	Normal eye	2.29±0.03	2.16±0.04	2.47±0.04	2.32±0.05	2.63±0.04	2.45±0.04	2.80±0.04	2.56±0.08	2.91±0.05	2.65±0.06
Vitreous chamber	Treated eye	6.61±0.30***	6.64±0.35***	6.41±0.35***	6.49±0.25*	6.16±0.25	7.09±0.25	6.38±0.18	7.64±0.29	6.59±0.39	7.98±0.38
depth (mm)	Normal eye	5.44±0.19	5.79±0.19	5.62±0.21	6.36±0.19	6.12±0.16	6.97±0.26	6.41±0.27	7.46±0.31	6.86±0.13	7.87±0.39
Axial length (mm)	Treated eye	11.00±0.39** *	10.62±0.47** *	11.15±0.77** *	10.61±0.35*	11.08±0.54**	11.45±0.27	11.47±0.26*	12.23±0.31	11.80±0.24	12.83±0.42
	Normal eye	9.19±0.19	9.40±0.21	9.72±0.23	10.32±0.26	10.46±0.23	11.30±0.29	11.08±0.30	12.06±0.35	11.76±0.20	12.72±0.38

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.2.1. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 17 both groups).

Ocular parameter		Week 1		Week 2		Week 3		Week 4	
		Female	Male	Female	Male	Female	Male	Female	Male
Refraction (D)	Treated eye	–	–	–21.3±9.2***	–20.7±8.7***	–6.3±8.5**	–7.4±8.5**	–0.3±5.1	–4.4±2.1*
	Normal eye	+2.8±1.3	+3.5±1.3	+3.2±1.2	+1.8±0.8	+1.5±1.2	+1.8±0.8	–1.2±4.7	+1.6±0.9
Corneal power	Treated eye	–	–	113.3±4.2	110.4±5.4	108.5±5.2*	105.3±4.8	101.4±5.8	97.8±5.1
(D)	Normal eye	120.4±2.4	120.8±4.2	110.8±4.0	110.0±4.0	101.9±3.5	101.5±3.4	95.9±3.9	94.5±3.3
Anterior chamber	Treated eye	1.19±0.28	1.24±0.22	1.90±0.28**	2.00±0.49**	2.24±0.44**	2.48±0.80**	2.35±0.50**	2.35±0.60**
depth (mm)	Normal eye	1.25±0.07	1.32±0.08	1.45±0.06	1.50±0.06	1.65±0.06	1.66±0.05	1.83±0.34	1.78±0.06
Axial lens	Treated eye	2.09±0.08	2.08±0.05	2.30±0.06	2.30±0.06	2.46±0.07	2.46±0.07	2.62±0.09	2.64±0.04
thickness (mm)	Normal eye	2.07±0.06	2.06±0.05	2.30±0.06	2.29±0.05	2.48±0.07	2.49±0.05	2.65±0.08	2.65±0.04
Vitreous chamber	Treated eye	5.81±0.31***	5.96±0.34***	6.55±0.62***	6.72±0.67***	6.23±0.58**	6.52±0.74**	6.14±0.46	6.46±0.74
depth (mm)	Normal eye	5.08±0.27	5.08±0.15	5.49±0.32	5.56±0.50	5.82±0.15	5.90±0.19	6.22±0.20	6.23±0.20
Axial length (mm)	Treated eye	9.10±0.42***	9.29±0.50***	10.76±0.84***	11.02±0.91***	10.93±0.96***	11.46±0.91**	11.11±0.83*	11.45±1.1*
	Normal eye	8.41±0.32	8.45±0.19	9.28±0.35	9.35±0.53	9.97±0.19	10.06±0.24	10.69±0.39	10.66±0.25

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.2.2. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 17 both groups).

Ocular parameter		Week 5		Week 6		Week 7		Week 8	
		Female	Male	Female	Male	Female	Male	Female	Male
Refraction (D)	Treated eye	−1.4±4.0	−1.8±6.0	+0.4±1.3	−1.7±5.7	+1.0±0.9	+0.4±2.2	+0.7±0.6	+0.8±0.7
	Normal eye	−1.3±0.7	+1.3±0.6	+1.2±0.7	+0.9±0.7	+0.7±0.8	+1.0±0.5	+0.5±0.6	+1.0±0.6
Corneal power	Treated eye	95.2±5.2	92.5±3.6	88.5±4.5	87.6±5.4	87.5±4.1	84.3±3.2	83.9±4.5	81.3±4.6
(D)	Normal eye	91.4±2.9	90.2±2.6	86.2±3.1	85.0±3.4	84.3±2.4	83.3±2.4	80.8±2.7	80.0±2.5
Anterior chamber	Treated eye	2.24±0.28*	2.58±1.0*	2.27±0.51	2.61±1.06	2.31±0.44	2.77±1.1	2.32±0.45	2.78±1.2
depth (mm)	Normal eye	1.87±0.50	1.89±0.05	1.97±0.11	2.01±0.09	2.04±0.10	2.14±0.18	2.16±0.15	2.20±0.07
Axial lens	Treated eye	2.76±0.07	2.77±0.05	2.89±0.07	2.90±0.05	2.97±0.10	2.99±0.05	3.08±0.10	3.07±0.07
thickness (mm)	Normal eye	2.79±0.07	2.79±0.04	2.92±0.07	2.92±0.05	3.02±0.08	3.03±0.05	3.11±0.08	3.11±0.06
Vitreous chamber	Treated eye	6.35±0.33	6.68±0.75	6.53±0.27	6.92±0.67	6.73±0.40	7.16±0.79	6.99±0.32	7.40±0.74
depth (mm)	Normal eye	6.45±0.21	6.54±0.22	6.73±0.27	6.78±0.26	6.89±0.26	7.04±0.30	7.09±0.35	7.29±0.25
Axial length (mm)	Treated eye	11.36±0.72	12.03±1.66	11.69±0.54	12.44±1.66	12.01±0.53	12.93±1.8	12.38±0.50	13.26±1.8
	Normal eye	11.12±0.29	11.23±0.25	11.61±0.35	11.72±0.32	11.96±0.33	12.22±0.38	12.36±0.38	12.60±0.33

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.3.1. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 9, 8).

Ocular parameter		Week 1		Week 2		Week 3		Week 4	
		RT	LT	RT	LT	RT	LT	RT	LT
Refraction (D)	Treated eye	–	–	–23.7±4.5***	–18.1±9.6***	–10.8±6.5**	–4.4±8.9	–5.3±8.1	–3.6±9.1
	Normal eye	+3.1±1.5	+3.7±1.3	+2.6±1.0	+1.1±4.2	+1.8±0.7	+1.7±0.9	+1.82±0.7	+1.4±0.9
Corneal power	Treated eye	–	–	111.0±4.9	109.9±6.1	104.3±4.0	106.1±5.5	98.6±3.8	97.1±6.1
(D)	Normal eye	121.4±4.5	120.2±4.4	110.0±3.3	110.0±4.7	102.3±3.5	100.7±3.4	95.3±3.2	93.7±3.3
Anterior chamber	Treated eye	1.22±0.28	1.27±0.22	1.90±0.28**	2.09±0.62**	2.41±0.47*	2.53±1.0	2.42±0.61	2.29±0.62
depth (mm)	Normal eye	1.29±0.07	1.35±0.04	1.46±0.07	1.52±0.06	1.64±0.04	1.69±0.05	1.77±0.05	1.79±0.07
Axial lens	Treated eye	2.08±0.05	2.08±0.05	2.29±0.04	2.31±0.07	2.45±0.08	2.47±0.06	2.65±0.04	2.63±0.05
thickness (mm)	Normal eye	2.05±0.05	2.06±0.05	2.28±0.05	2.31±0.05	2.48±0.05	2.50±0.05	2.64±0.04	2.66±0.05
Vitreous chamber	Treated eye	6.01±0.37***	5.92±0.38	7.06±0.44***	6.40±0.71	6.70±0.89**	6.39±0.61	6.52±0.87	6.40±0.66
depth (mm)	Normal eye	5.06±0.16	5.08±0.14	5.42±0.27	5.68±0.63	5.87±0.24	5.93±0.15	6.19±0.25	6.26±0.14
Axial length (mm)	Treated eye	9.31±0.56***	9.27±0.48***	11.25±0.60***	10.80±1.1	11.53±1.3**	11.39±1.5	11.59±1.4	11.31±1.1
	Normal eye	8.40±0.24	8.50±0.13	9.16±0.32	9.51±0.65	9.99±0.30	10.12±0.17	10.60±0.30	10.71±0.19

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.2.3.2. Ocular parameters of treated and normal eyes, lid suture for 2 weeks (mean ± SD, n = 9, 8).

Ocular parameter		Week 5		Week 6		Week 7		Week 8	
		RT	LT	RT	LT	RT	LT	RT	LT
Refraction (D)	Treated eye	–2.2±6.6	–1.4±5.8	–2.3±5.9	–1.2±5.9	+1.0±0.9	–0.2±2.8	+0.7±0.9	+0.8±0.5
	Normal eye	+1.4±0.7	+1.2±0.5	+0.9±0.7	+0.9±0.7	+1.7±0.6	+0.9±0.6	+1.0±0.7	+0.9±0.5
Corneal power	Treated eye	93.2±2.5	91.8±4.3	89.2±2.2	86.1±5.5	85.9±2.0	82.8±3.4	83.7±2.3	79.1±5.1
(D)	Normal eye	90.7±2.6	89.7±2.7	85.7±3.1	84.4±3.7	83.6±2.5	83.1±2.5	81.0±2.6	79.1±2.3
Anterior chamber	Treated eye	2.46±0.60*	2.68±1.2*	2.51±0.60*	2.71±1.3*	2.55±0.59*	2.97±1.3	2.58±0.55	2.97±1.6
depth (mm)	Normal eye	1.88±0.05	1.91±0.04	2.02±0.11	2.01±0.08	2.08±0.07	2.20±0.07	2.20±0.08	2.20±0.07
Axial lens	Treated eye	2.77±0.04	2.76±0.06	2.90±0.06	2.90±0.04	3.00±0.06	2.99±0.03	3.09±0.08	3.06±0.07
thickness (mm)	Normal eye	2.80±0.05	2.79±0.03	2.91±0.05	2.93±0.02	3.02±0.04	3.05±0.07	3.10±0.05	3.12±0.08
Vitreous chamber	Treated eye	6.67±0.86	6.69±0.70	6.85±0.59	6.99±0.78	7.00±0.52	7.30±1.0	7.19±0.46	7.58±0.92
depth (mm)	Normal eye	6.53±0.27	6.56±0.17	6.81±0.32	6.76±0.22	7.01±0.35	7.07±0.28	7.23±0.48	7.34±0.26
Axial length (mm)	Treated eye	11.90±1.4*	12.14±1.9*	12.27±1.1	12.59±2.0*	12.56±1.0	13.26±2.3	12.85±0.88	13.64±2.4
	Normal eye	11.20±0.29	11.26±0.22	11.75±0.41	11.70±0.25	12.11±0.41	12.31±0.31	12.53±0.32	12.66±0.35

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.3.1. Ocular parameters of treated and normal eyes, at day 5 (mean ± SD, n = 6 all groups).

Ocular parameter		Treatment group				
		CO	20	40	60	120
Refraction (D)	Treated eye	−10.0±3.3***	−3.2±2.1**	−1.3±1.8**	−1.5±2.9**	−1.4±0.9**
	Normal eye	+2.5±0.9	+1.7±1.6	+2.5±1.4	+2.3±1.1	+2.5±1.0
Corneal power	Treated eye	120.5±1.2*	116.6±2.3	118.1±3.0	117.9±1.5	117.8±3.7
(D)	Normal eye	118.0±2.4	117.5±2.1	117.5±2.6	118.6±2.3	117.9±1.6
Anterior chamber	Treated eye	1.14±0.05*	1.12±0.05	1.11±0.04	1.15±0.05	1.11±0.03
depth (mm)	Normal eye	1.11±0.03	1.12±0.01	1.11±0.03	1.14±0.04	1.10±0.02
Axial lens	Treated eye	1.87±0.03	1.87±0.01	1.85±0.03	1.83±0.02	1.85±0.04
thickness (mm)	Normal eye	1.85±0.03	1.88±0.02	1.85±0.04	1.83±0.02	1.86±0.01
Vitreous chamber	Treated eye	5.32±0.16***	5.26±0.11**	5.23±0.07**	5.21±0.09**	5.12±0.13**
depth (mm)	Normal eye	4.85±0.19	4.97±0.08	4.98±0.10	4.98±0.10	4.86±0.17
Axial length (mm)	Treated eye	8.33±0.18***	8.25±0.13**	8.18±0.08**	8.20±0.14**	8.10±0.15**
	Normal eye	7.82±0.20	7.96±0.09	7.94±0.07	7.95±0.13	7.83±0.16

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.3.2. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 8 all groups).

Ocular parameter		Treatment group		
		CO	am	pm
Refraction (D)	Treated eye	−19.4±7.7***	−4.2±3.1***	−3.7±3.6***
	Normal eye	+1.5±1.5	+1.5±2.1	+1.7±2.2
Corneal power	Treated eye	113.4±4	109.1±3.4	110.0±5
(D)	Normal eye	112.1±1.4	110.2±3.6	110.2±2.9
Anterior chamber	Treated eye	1.41±0.15***	1.37±0.15***	1.41±0.17***
depth (mm)	Normal eye	1.28±0.03	1.27±0.04	1.25±0.04
Axial lens	Treated eye	2.12±0.06	2.08±0.06	2.06±0.04
thickness (mm)	Normal eye	2.11±0.04	2.08±0.05	2.08±0.04
Vitreous chamber	Treated eye	5.98±0.38***	5.65±0.18***	5.59±0.23***
depth (mm)	Normal eye	5.22±0.14	5.31±0.15	5.27±0.18
Axial length	Treated eye	9.51±0.42***	9.10±0.21***	9.07±0.28***
(mm)	Normal eye	8.61±0.16	8.67±0.18	8.62±0.19

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.3.4.1. Ocular parameters of treated and normal eyes, day 5 (mean ± SD).

Ocular		Myopic defocus					Hyperopic defocus				
parameter	Daily NV	0 hrs/day	3	6	9	11	0 hrs/day	3	6	9	11
Refraction (D)	Treated eye	+8.7±2.9***	+8.4±2.6***	+7.0±0.8***	+6.3±2.2***	+3.3±1.3**	+1.1±0.7*	+2.3±1.9	+3.5±1.7	+2.9±1.9	+2.2±2.3
	Normal eye	+0.4±1.9	+0.8±1.0	+0.9±1.8	+1.4±1.7	+0.8±1.8	+2.8±0.8	+1.1±1.5	+2.1±1.3	+1.7±2.1	+1.3±2.4
Corneal power	Treated eye	116.3±1.7	117.1±3.2	116.0±2.2	118.6±4.1	116.9±2.5	118.2±3.0	116.1±4.2	116.4±2.0	115.6±3.3	117.8±2.6
(D)	Normal eye	118.0±2.2	117.5±1.8	117.0±2.2	116.7±2.9	116.4±3.4	118.8±4.0	118.6±2.7	115.2±2.2	118.2±3.2	119.2±3.6
Anterior chamber	Treated eye	1.13±0.08	1.10±0.04	1.09±0.02	1.08±0.05	1.10±0.04	1.10±0.04	1.11±0.02	1.10±0.04	1.12±0.03	1.14±0.04
depth (mm)	Normal eye	1.10±0.03	1.11±0.04	1.10±0.03	1.09±0.05	1.13±0.03	1.10±0.02	1.09±0.03	1.10±0.03	1.10±0.04	1.13±0.04
Axial lens	Treated eye	1.86±0.03	1.86±0.04	1.87±0.04	1.88±0.05	1.90±0.03	1.88±0.03	1.91±0.06	1.91±0.04	1.90±0.03	1.86±0.03
thickness (mm)	Normal eye	1.87±0.03	1.87±0.04	1.88±0.04	1.89±0.03	1.89±0.03	1.89±0.02	1.92±0.04	1.91±0.04	1.91±0.04	1.87±0.03
Vitreous chamber	Treated eye	4.72±0.22** *	4.84±0.21** *	4.87±0.13** *	4.81±0.14**	4.99±0.11**	5.15±0.04**	5.09±0.09	5.07±0.09	4.97±0.09	5.02±0.15
depth (mm)	Normal eye	5.01±0.04	5.06±0.12	5.07±0.09	4.96±0.09	5.10±0.08	5.00±0.05	5.08±0.11	5.08±0.13	5.00±0.06	5.07±0.11
Axial length (mm)	Treated eye	7.71±0.22** *	7.80±0.22** *	7.85±0.14** *	7.78±0.16** *	7.99±0.14**	8.14±0.06	8.12±0.10	8.08±0.09	7.99±0.10	8.02±0.18
	Normal eye	8.00±0.06	8.05±0.14	8.06±0.10	7.93±0.14	8.12±0.09	8.00±0.06**	8.10±0.19	8.10±0.13	8.02±0.06	8.08±0.12

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.3.4.2. Ocular parameters of treated and normal eyes, day 10 (mean ± SD).

Ocular		Myopic defocus					Hyperopic defocus				
parameter	Daily NV	0 hrs/day	3	6	9	11	0 hrs/day	3	6	9	11
Refraction (D)	Treated eye	+8.0±1.7***	+6.3±1.4***	+3.8±1.8**	+3.5±1.4**	+2.3±1.1*	−3.7±2.7*	+1.0±1.7	+2.1±2.3	+1.0±3.5	+1.7±0.9
	Normal eye	−0.3±1.3	+0.5±1.5	+0.6±1.2	+0.4±1.8	−0.6±1.3	+2.4±0.9	+1.1±1.9	+0.6±1.5	+0.9±2.7	+0.8±1.0
Corneal power	Treated eye	107.3±2.7**	109.2±2.0*	111.0±1.9	110.6±2.7	110.6±2.5	110.3±2.7*	111.4±1.9	110.5±2.0	113.3±2.1	111.9±2.9
(D)	Normal eye	113.5±2.6	112.0±1.5	111.7±1.2	111.4±2.9	110.0±2.6	112.5±1.7	111.6±1.6	112.4±1.6	112.2±1.1	112.5±2.3
Anterior chamber	Treated eye	1.19±0.07	1.19±0.04	1.23±0.05	1.21±0.07	1.21±0.04	1.25±0.04*	1.23±0.04*	1.20±0.06	1.25±0.03	1.24±0.03
depth (mm)	Normal eye	1.20±0.05	1.20±0.06	1.23±0.03	1.21±0.06	1.20±0.05	1.23±0.06	1.20±0.04	1.22±0.05	1.23±0.03	1.23±0.03
Axial lens	Treated eye	2.06±0.03	2.06±0.04	2.07±0.05	2.10±0.03	2.07±0.04	2.07±0.06	2.10±0.05	2.09±0.06	2.12±0.03	2.05±0.03
thickness (mm)	Normal eye	2.07±0.03	2.07±0.04	2.08±0.04	2.10±0.03	2.08±0.03	2.07±0.02	2.12±0.05	2.11±0.03	2.11±0.02	2.06±0.04
Vitreous chamber	Treated eye	4.97±0.15** *	5.00±0.20***	5.13±0.14**	5.03±0.14**	5.13±0.15**	5.33±0.05**	5.24±0.09*	5.22±0.10	5.10±0.03	5.14±0.18
depth (mm)	Normal eye	5.14±0.07	5.15±0.08	5.18±0.07	5.13±0.07	5.24±0.10	5.06±0.03	5.15±0.11	5.24±0.13	5.16±0.09	5.14±0.16
Axial length (mm)	Treated eye	8.23±0.16** *	8.20±0.14***	8.43±0.20*	8.34±0.20*	8.41±0.19*	8.65±0.09***	8.56±0.10**	8.52±0.12	8.47±0.05	8.43±0.19
	Normal eye	8.42±0.08	8.42±0.09	8.49±0.10	8.44±0.11	8.53±0.20	8.36±0.05	8.47±0.12	8.58±0.16	8.49±0.10	8.43±0.21

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.4.1. Ocular parameters of treated and normal eyes, day 11 (mean ± SD, n= 7 all groups except plano where n = 9).

Ocular		+10 D + CNS (myopic defocus)				−10 D + CNS (hyperopic defocus)				Plano + CNS
parameter	Daily NV	0 hrs/day	3	6	9	0 hrs/day	3	6	9	0
Refraction (D)	Treated eye	+12.0±4.4***	+7.5±2.8***	+6.6±0.6**	+4.5±1.1**	−4.8±1.5***	+0.5±1.9*	+1.8±1.6	+2.1±2.9	+1.3±0.8
	Normal eye	+1.4±1.8	+1.6±1.4	+2.1±1.6	+1.8±0.6	+1.5±1.4	+2.9±2.3	+2.1±0.8	+2.4±0.7	+1.9±0.4
Corneal power	Treated eye	106.3±3.4*	104.4±1.6*	107.4±4.2	111.4±3.5	111.7±4.4	109.5±2.0	110.6±1.9	110.5±2.7	109.2±3.7
(D)	Normal eye	110.9±2.4	108.8±1.3	110.1±2.6	111.2±1.8	110.1±1.5	110.7±4.4	107.9±4.6	110.5±1.7	109.9±4.0
Anterior chamber	Treated eye	1.15±0.08**	1.12±0.06**	1.21±0.05	1.27±0.09	1.32±0.10**	1.24±0.09	1.23±0.08	1.24±0.05	1.31±0.09*
depth (mm)	Normal eye	1.23±0.04	1.21±0.05	1.24±0.05	1.24±0.04	1.23±0.04	1.23±0.05	1.23±0.03	1.23±0.03	1.24±0.03
Axial lens	Treated eye	2.04±0.04***	2.06±0.04***	1.99±0.05***	2.02±0.05***	2.09±0.05**	2.04±0.03**	1.98±0.05**	2.02±0.05*	1.97±0.03**
thickness (mm)	Normal eye	2.12±0.03	2.11±0.03	2.05±0.04	2.09±0.05	2.13±0.03	2.10±0.03	2.05±0.04	2.06±0.05	2.05±0.03
Vitreous chamber	Treated eye	4.85±0.26***	5.18±0.20***	5.06±0.17**	5.15±0.24**	5.59±0.12***	5.34±0.20**	5.32±0.18**	5.16±0.18*	5.29±0.25*
depth (mm)	Normal eye	5.14±0.10	5.24±0.07	5.14±0.17	5.07±0.09	5.18±0.24	5.15±0.21	5.15±0.14	5.05±0.09	5.15±0.12
Axial length (mm)	Treated eye	8.03±0.27***	8.44±0.24**	8.27±0.19**	8.44±0.36	9.01±0.28***	8.62±0.25**	8.54±0.20*	8.43±0.20*	8.58±0.38*
	Normal eye	8.48±0.10	8.57±0.10	8.42±0.16	8.39±0.14	8.54±0.23	8.48±0.21	8.43±0.15	8.34±0.12	8.44±0.12

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.2.1. Ocular parameters of treated and control eyes (mean ± SD, n = 7, 8, 7).

Ocular parameter		Week 1			Week 2			Week 3		
		Blue	White	Red	Blue	White	Red	Blue	White	Red
Refraction (D)	Treated eye	−7.1±2.9***	−6.7±4.1***	−2.1±3.6***	−1.8±1.5	−2.0±1.4	−0.7±1.6	−1.0±0.9	−1.3±1.5	−0.5±1.3
	Control eye	+0.2±0.9	+1.3±0.9	+0.2±1.3	−0.7±0.9	−0.9±1.2	+0.8±1.1	+0.2±0.7	−0.7±0.7	+0.5±1.4
Corneal power	Treated eye	113.4±2.1	111.8±1.2	115.5±2.0	102.1±2.1	100.3±1.5	101.0±2.1	94.0±1.9	93.5±1.6	94.6±2.3
(D)	Control eye	111.8±2.2	111.4±1.5	113.7±1.3	102.6±2.4	100.8±1.7	103.8±1.2	93.9±2.0	94.2±1.8	95.3±2.2
Anterior chamber	Treated eye	1.25±0.06***	1.21±0.08	1.18±0.02	1.38±0.09	1.33±0.08	1.32±0.07	1.57±0.06	1.51±0.08	1.52±0.09
depth (mm)	Control eye	1.19±0.06	1.22±0.04	1.19±0.02	1.42±0.06	1.34±0.09	1.35±0.07	1.56±0.06	1.52±0.09	1.55±0.09
Axial lens	Treated eye	2.08±0.04	2.06±0.04	2.05±0.02	2.35±0.02	2.30±0.04	2.30±0.02	2.50±0.02	2.46±0.04	2.50±0.03
thickness (mm)	Control eye	2.09±0.04	2.06±0.04	2.05±0.02	2.35±0.02	2.31±0.04	2.30±0.02	2.50±0.02	2.46±0.04	2.50±0.03
Vitreous chamber	Treated eye	5.70±0.12***	5.68±0.19***	5.47±0.12***	5.91±0.15***	6.10±0.13***	5.98±0.11	6.33±0.16	6.45±0.15	6.47±0.15
depth (mm)	Control eye	5.41±0.15	5.45±0.12	5.28±0.06	5.83±0.17	5.96±0.12	5.94±0.12	6.25±0.17	6.41±0.17	6.43±0.17
Axial length (mm)	Treated eye	9.03±0.16***	8.95±0.25***	8.70±0.12***	9.64±0.14	9.74±0.13***	9.60±0.12	10.40±0.18	10.42±0.16	10.49±0.13
	Control eye	8.68±0.20	8.73±0.14	8.52±0.07	9.60±0.18	9.64±0.12	9.60±0.12	10.31±0.19	10.39±0.19	10.47±0.15

*Differences between treated and control eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.2.2. Ocular parameters of treated and control eyes (mean ± SD, n = 7, 8, 7).

Ocular parameter		Week 4			Week 5			Week 6		
		Blue	White	Red	Blue	White	Red	Blue	White	Red
Refraction (D)	Treated eye	–0.5±0.9	–0.5±0.7	–0.1±0.9	–0.8±0.9	–0.7±0.7	–0.1±0.7	–0.6±0.5	+0.06±0.6	+0.8±0.3
	Control eye	+0.2±0.6	–0.3±0.5	+0.2±0.6	–0.7±0.6	–0.7±0.2	–0.05±1.2	–0.3±0.4	+0.02±0.4	+0.8±0.4
Corneal power	Treated eye	88.2±1.5	87.4±1.7	88.3±2.4	81.7±1.7	82.5±1.7	82.2±2.3	78.4±1.5	78.4±1.5	116.7±2.4
(D)	Control eye	88.9±1.9	88.4±1.8	88.2±2.0	82.6±1.9	83.3±1.6	82.4±2.2	78.7±1.5	78.8±1.5	117.1±2.1
Anterior chamber	Treated eye	1.71±0.11	1.72±0.07	1.68±0.09	1.85±0.11	1.87±0.08	1.77±0.09	1.97±0.07	2.04±0.08	1.94±0.11
depth (mm)	Control eye	1.69±0.09	1.71±0.09	1.69±0.07	1.83±0.09	1.86±0.10	1.77±0.10	1.95±0.14	2.02±0.09	1.94±0.10
Axial lens	Treated eye	2.68±0.06	2.65±0.02	2.68±0.03	2.83±0.02	2.80±0.04	2.80±0.02	2.95±0.02	2.92±0.04	2.91±0.03
thickness (mm)	Control eye	2.68±0.02	2.65±0.02	2.68±0.04	2.83±0.02	2.81±0.04	2.80±0.02	2.95±0.02	2.92±0.04	2.90±0.03
Vitreous chamber	Treated eye	6.87±0.19	6.82±0.16	6.87±0.20	7.29±0.19	7.21±0.16	7.23±0.21	7.66±0.20	7.55±0.19	7.58±0.26
depth (mm)	Control eye	6.82±0.18	6.79±0.16	6.82±0.21	7.24±0.19	7.18±0.16	7.23±0.22	7.69±0.21	7.54±0.19	7.51±0.26
Axial length (mm)	Treated eye	11.26±0.17	11.20±0.20	11.27±0.15	11.97±0.19	11.88±0.19	11.80±0.18	12.51±0.21	12.51±0.22	12.41±0.21
	Control eye	11.19±0.18	11.16±0.19	11.19±0.17	11.91±0.19	11.85±0.19	11.77±0.21	12.606±0.26	12.48±0.23	12.45±0.17

*Differences between treated and control eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.2.3. Ocular parameters of treated and control eyes, week 6 (mean±SD, n = 7, 8, 7).

Ocular parameter		Treatment group		
		Blue	White	Red
External axial length (mm)	Treated eye	12.95±0.24*	12.67±0.22	12.91±0.22
	Control eye	12.58±0.30	12.61±0.28	12.80±0.26
Equatorial diameter (mm)	Treated eye	16.51±.0.26	16.64±0.28	17.01±.0.36
	Control eye	16.49±0.28	16.60±0.32	16.99±0.40
Ratio AL/EQD	Treated eye	0.78±0.01	0.76±0.02	0.76±0.01
	Control eye	0.76±0.01	0.76±0.01	0.75±0.01

*Differences between treated and control eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.3.1. Ocular parameters of treated and normal eyes, at day 5 (mean ± SD, n = 7, 8, 10, 10, 10, 10).

Ocular parameter		Treatment group					
		CO	BW	DW	Blue	Yellow	Red
Refraction (D)	Treated eye	−9.6±2.1***	−2.0±0.9***	−1.4±1.3***	−1.6±1.0***	−1.8±0.9***	−2.3±2.3***
	Normal eye	+2.4±0.8	+2.4±0.9	+3.0±1.1	+3.2±0.9	+3.5±1.0	+4.0±1.2
Corneal power	Treated eye	120.0±1.1*	118.4±2.0	117.9±3.1	116.5±1.9	116.5±2.5	116.7±2.4
(D)	Normal eye	118.5±2.2	119.5±4.4	117.7±2.6	115.9±1.8	116.6±2.6	117.1±2.1
Anterior chamber	Treated eye	1.14±0.04**	1.12±0.06**	1.13±0.04	1.12±0.06**	1.15±0.04*	1.12±0.04**
depth (mm)	Normal eye	1.09±0.02	1.09±0.05	1.11±0.03	1.08±0.03	1.12±0.03	1.08±0.04
Axial lens	Treated eye	1.85±0.03	1.88±0.02	1.85±0.03	1.88±0.04	1.84±0.03	1.86±0.03
thickness (mm)	Normal eye	1.85±0.03	1.87±0.03	1.85±0.03	1.88±0.03	1.85±0.03	1.86±0.04
Vitreous chamber	Treated eye	5.33±0.10***	5.05±0.09***	5.21±0.14***	5.22±0.10***	5.18±0.11***	5.18±0.11***
depth (mm)	Normal eye	4.84±0.14	4.85±0.09	4.99±0.11	4.99±0.07	4.93±0.10	4.87±0.09
Axial length (mm)	Treated eye	8.32±0.10***	8.05±0.11***	8.20±0.12***	8.22±0.15***	8.17±0.12***	8.17±0.14***
	Normal eye	7.77±0.11	7.80±0.11	7.95±0.11	7.96±0.11	7.90±0.10	7.81±0.14

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.3.2. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 6, 6, 8, 8, 7, 6).

Ocular parameter		Treatment group					
		CO	BW	DW	Blue	Yellow	Red
Refraction (D)	Treated eye	−19.7±5.1***	−4.7±2.3***	−8.3±8.0***	−7.9±6.7***	−4.2±3.2***	−3.5±2.9***
	Normal eye	+1.8±1.4	+0.9±0.9	+1.4±1.3	+2.7±1.4	+2.5±1.8	+2.4±1.1
Corneal power	Treated eye	113.3±2.4*	113.4±1.9	111.7±5.1	110.1±1.7	113.3±4.8	113.3±5.1
(D)	Normal eye	111.7±2.9	114.1±1.3	112.5±1.7	109.7±3.4	113.2±4.1	113.2±4.3
Anterior chamber	Treated eye	1.33±0.10***	1.31±0.09**	1.27±0.12*	1.32±0.06***	1.27±0.09*	1.27±0.09*
depth (mm)	Normal eye	1.22±0.04	1.26±0.03	1.23±0.05	1.23±0.07	1.23±0.05	1.23±0.05
Axial lens	Treated eye	2.05±0.03	2.07±0.03	2.05±0.04	2.07±0.03	2.03±0.03	2.03±0.02
thickness (mm)	Normal eye	2.04±0.03	2.07±0.03	2.06±0.05	2.08±0.03	2.03±0.03	2.03±0.02
Vitreous chamber	Treated eye	5.86±0.21***	5.29±0.11***	5.60±0.24***	5.62±0.21***	5.44±0.19***	5.33±0.09***
depth (mm)	Normal eye	5.02±0.16	4.94±0.13	5.11±0.08	5.09±0.06	5.06±0.11	4.96±0.09
Axial length (mm)	Treated eye	9.24±0.19***	8.68±0.12***	8.94±0.28***	9.02±0.22***	8.74±0.17***	8.59±0.10***
	Normal eye	8.28±0.16	8.27±0.11	8.40±0.05	8.41±0.07	8.32±0.10	8.18±0.10

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.5.3.3. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 6, 6, 8, 8, 7, 6).

Ocular parameter		Treatment group					
		CO	BW	DW	Blue	Yellow	Red
External axial length	Treated eye	9.90±0.32***	9.54±0.26***	9.67±0.30**	9.73±0.28**	9.50±0.20**	9.51±0.20**
(mm)	Normal eye	9.00±0.20	9.03±0.06	9.24±0.17	9.28±0.17	9.05±0.19	9.09±0.11
Equatorial diameter	Treated eye	12.21±.0.45**	12.08±0.18**	12.18±.0.30	12.30±.0.33	12.14±.0.17*	11.99±0.26
(mm)	Normal eye	11.81±0.19	11.76±0.15	12.00±0.21	12.17±0.25	11.84±0.26	11.75±0.19
Ratio AL/EQD	Treated eye	0.810±0.02**	0.789±0.01*	0.794±0.01*	0.791±0.02*	0.782±0.01	0.793±0.02
	Normal eye	0.762±0.01	0.768±0.01	0.770±0.01	0.764±0.01	0.764±0.02	0.773±0.01
Wet eye weight (g)	Treated eye	0.71±0.06***	0.66±0.03**	0.69±0.05*	0.69±0.03*	0.67±0.04*	0.64±0.04*
	Normal eye	0.62±0.04	0.60±0.02	0.64±0.02	0.64±0.03	0.61±0.02	0.59±0.02

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.1.1. Ocular parameters of treated and normal eyes, at day 5 (mean ± SD, n = 7, 7, 10, 10, 10, 10).

Ocular parameter		Treatment group					
		CO	NV	High	Medium	Low	Mix
Refraction (D)	Treated eye	−9.5±2.8***	−1.5±2.0***	−2.5±2.2***	−2.3±2.5***	−4.3±2.3***	−2.6±1.3***
	Normal eye	+2.5±0.9	+2.2±1.4	+2.4±1.8	+2.1±1.5	+2.6±1.3	+2.6±1.3
Corneal power	Treated eye	119.1±1.8*	116.9±1.6	117.0±3.5	117.7±2.9	116.6±1.8	118.0±2.7
(D)	Normal eye	117.6±2.2	117.8±2.8	118.0±1.6	118.1±2.1	116.9±1.9	117.9±2.1
Anterior chamber	Treated eye	1.18±0.05***	1.14±0.05*	1.12±0.05*	1.14±0.03	1.14±0.04	1.13±0.04*
depth (mm)	Normal eye	1.11±0.03	1.11±0.05	1.09±0.04	1.12±0.03	1.11±0.03	1.10±0.05
Axial lens	Treated eye	1.86±0.03	1.87±0.01	1.85±0.03	1.85±0.04	1.85±0.02	1.87±0.04
thickness (mm)	Normal eye	1.85±0.03	1.88±0.01	1.86±0.04	1.86±0.05	1.85±0.02	1.87±0.03
Vitreous chamber	Treated eye	5.34±0.16***	5.14±0.09***	5.18±0.14***	5.26±0.12***	5.30±0.13***	5.22±0.18***
depth (mm)	Normal eye	4.85±0.19	4.95±0.08	4.90±0.11	4.95±0.13	4.97±0.14	4.90±0.17
Axial length (mm)	Treated eye	8.37±0.18***	8.15±0.09***	8.16±0.18***	8.25±0.12***	8.30±0.16***	8.22±0.17***
	Normal eye	7.82±0.20	7.94±0.11	7.85±0.15	7.93±0.15	7.94±0.16	7.86±0.16

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.1.2. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 7, 6, 9, 7, 8, 7).

Ocular parameter		Treatment group					
		CO	NV	High	Medium	Low	Mix
Refraction (D)	Treated eye	−17.3±7.0***	−4.7±3.1***	−4.9±2.4***	−3.7±3.7***	−7.0±4.2*	−6.1±2.8***
	Normal eye	+1.8±0.58	+1.0±1.4	+1.8±1.4	+2.3±1.2	+1.3±0.7	+1.7±0.9
Corneal power	Treated eye	115.5±2.5*	111.7±2.0	111.6±3.0	111.8±2.8	110.6±1.7	113.5±2.4
(D)	Normal eye	112.0±3.2	111.7±1.5	111.8±2.6	112.8±3.1	112.1±1.4	113.0±1.9
Anterior chamber	Treated eye	1.34±0.15***	1.31±0.08**	1.25±0.07	1.30±0.10	1.28±0.08	1.30±0.08*
depth (mm)	Normal eye	1.21±0.06	1.24±0.04	1.21±0.04	1.26±0.05	1.24±0.03	1.24±0.06
Axial lens	Treated eye	2.05±0.05	2.03±0.05	2.04±0.04	2.03±0.06	2.03±0.03	2.05±0.06
thickness (mm)	Normal eye	2.05±0.05	2.03±0.04	2.05±0.05	2.04±0.06	2.04±0.03	2.06±0.06
Vitreous chamber	Treated eye	5.84±0.40***	5.41±0.20***	5.51±0.14***	5.52±0.17***	5.61±0.25*	5.52±0.21***
depth (mm)	Normal eye	5.01±0.21	5.05±0.12	5.03±0.14	5.12±0.16	5.09±0.11	5.04±0.17
Axial length (mm)	Treated eye	9.24±0.47***	8.75±0.20***	8.79±0.17***	8.84±0.12***	8.92±0.28*	8.86±0.22***
	Normal eye	8.27±0.24	8.33±0.11	8.28±0.15	8.39±0.16	8.36±0.10	8.33±0.17

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.1.3. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 7, 6, 9, 7, 8, 7).

Ocular parameter		Treatment group					
		CO	NV	High	Medium	Low	Mix
External axial length	Treated eye	9.95±0.38***	9.70±0.29***	9.39±0.26*	9.55±0.38*	9.53±0.30**	9.40±0.17**
(mm)	Normal eye	9.11±0.26	9.10±0.28	9.13±0.19	9.22±0.13	9.13±0.22	9.16±0.09
Equatorial diameter	Treated eye	12.27±.0.37**	12.22±.011**	12.04±.0.13	12.27±.0.18**	12.17±.0.17**	12.19±.032*
(mm)	Normal eye	11.94±0.30	11.82±0.12	11.84±0.17	11.97±0.17	11.81±0.20	11.83±0.31
Ratio AL/EQD	Treated eye	0.809±0.03**	0.793±0.03*	0.786±0.02	0.778±0.03	0.782±0.03	0.780±0.02
	Normal eye	0.762±0.01	0.767±0.02	0.771±0.01	0.771±0.01	0.770±0.01	0.766±0.01
Wet eye weight (g)	Treated eye	0.72±0.06***	0.69±0.01**	0.65±0.03	0.69±0.04*	0.68±0.04	0.66±0.04
	Normal eye	0.62±0.05	0.62±0.03	0.61±0.02	0.63±0.02	0.68±0.04	0.61±0.04

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.2.1. Ocular parameters of treated and normal eyes, at day 5 (mean ± SD, n = 7, 7, 10, 10, 10, 10, 10).

Ocular parameter		Treatment group						
		CO	NV	High	Mid	Low	High/Low	Mixed
Refraction (D)	Treated eye	−9.2±2.6***	−1.7±1.0***	−8.2±3.4***	−0.6±1.0***	−4.8±2.3***	−4.7±3.6***	−1.0±1.6***
	Normal eye	+2.4±0.98	+1.6±1.8	+2.7±0.8	+3.4±1.4	+2.5±1.4	+2.7±1.4	+3.2±1.4
Corneal power	Treated eye	120.2±2.0*	118.2±1.1	119.9±1.6**	117.9±2.9	118.7±2.8	118.4±3.0	118.3±2.1
(D)	Normal eye	117.8±2.1	118.4±1.2	116.6±2.7	117.7±2.0	117.8±2.0	117.7±1.8	118.8±2.1
Anterior chamber	Treated eye	1.14±0.05*	1.10±0.04	1.17±0.04***	1.10±0.04	1.13±0.04*	1.12±0.05**	1.11±0.04
depth (mm)	Normal eye	1.08±0.02	1.09±0.05	1.10±0.03	1.10±0.02	1.10±0.03	1.06±0.05	1.09±0.03
Axial lens	Treated eye	1.85±0.03	1.88±0.02	1.85±0.04	1.87±0.05	1.89±0.02	1.85±0.02	1.86±0.04
thickness (mm)	Normal eye	1.85±0.04	1.88±0.01	1.85±0.04	1.86±0.04	1.89±0.03	1.86±0.03	1.87±0.04
Vitreous chamber	Treated eye	5.32±0.10***	5.03±0.08***	5.28±0.13***	5.11±0.11***	5.23±0.13***	5.13±0.11***	5.09±0.07***
depth (mm)	Normal eye	4.86±0.13	4.86±0.10	4.89±0.09	4.94±0.11	4.89±0.11	4.78±0.16	4.87±0.07
Axial length (mm)	Treated eye	8.31±0.10***	8.01±0.11***	8.30±0.14***	8.07±0.13***	8.25±0.14***	8.11±0.15***	8.07±0.10***
	Normal eye	7.79±0.12	7.83±0.16	7.83±0.11	7.90±0.13	7.88±0.11	7.70±0.21	7.83±0.10

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.2.2. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 7, 7, 9, 8, 7, 7, 9).

Ocular parameter		Treatment group						
		CO	NV	High	Mid	Low	High/Low	Mix
Refraction (D)	Treated eye	−17.3±9.9***	−3.6±2.7***	−11.6±7.2***	−2.8±1.7***	−7.1±4.6***	−7.4±2.0***	−3.0±2.9***
	Normal eye	+2.3±1.7	+1.4±1.0	+2.0±2.0	+2.1±0.9	+1.7±1.1	+2.4±1.1	+2.0±0.9
Corneal power	Treated eye	113.5±1.6*	112.5±1.8	114.9±3.8*	111.5±2.7	111.8±4.3	112.0±3.0	112.0±1.9
(D)	Normal eye	111.1±1.5	112.7±2.2	112.0±2.6	112.9±2.4	111.5±2.0	111.6±1.4	112.9±2.2
Anterior chamber	Treated eye	1.33±0.05**	1.30±0.09*	1.39±0.14***	1.24±0.06	1.30±0.09*	1.28±0.08**	1.26±0.07*
depth (mm)	Normal eye	1.23±0.06	1.24±0.03	1.24±0.03	1.23±0.04	1.22±0.03	1.18±0.06	1.22±0.04
Axial lens	Treated eye	2.04±0.04	2.05±0.04	2.05±0.05	2.06±0.06	2.05±0.06	2.03±0.05	2.04±0.05
thickness (mm)	Normal eye	2.03±0.03	2.06±0.03	2.04±0.04	2.06±0.05	2.05±0.05	2.04±0.05	2.05±0.05
Vitreous chamber	Treated eye	5.79±0.33***	5.24±0.13***	5.63±0.40***	5.43±0.16***	5.64±0.19***	5.43±0.22***	5.34±0.21***
depth (mm)	Normal eye	5.03±0.15	4.95±0.12	5.05±0.13	5.08±0.18	5.08±0.10	4.96±0.13	5.06±0.14
Axial length (mm)	Treated eye	9.16±0.34***	8.59±0.12***	9.07±0.45***	8.73±0.18***	9.00±0.28***	8.74±0.22***	8.64±0.25***
	Normal eye	8.29±0.16	8.24±0.14	8.34±0.15	8.36±0.21	8.35±0.13	8.18±0.13	8.32±0.17

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

Table AII.6.2.3. Ocular parameters of treated and normal eyes, at day 10 (mean ± SD, n = 7, 7, 9, 8, 7, 7, 9).

Ocular parameter		Treatment group						
		CO	NV	High	Mid	Low	High/Low	Mix
External axial length	Treated eye	9.90±0.32***	9.41±0.36**	9.70±0.37**	9.46±0.24	9.68±0.36*	9.66±0.11*	9.46±0.30*
(mm)	Normal eye	9.03±0.20	9.04±0.08	9.21±0.17	9.22±0.26	9.18±0.18	9.19±0.16	9.15±0.24
Equatorial diameter	Treated eye	12.32±.0.30**	12.05±0.14**	12.18±.0.25*	12.16±.0.14*	12.24±.0.28*	12.13±.0.24*	12.07±.0.13*
(mm)	Normal eye	11.80±0.20	11.73±0.27	11.88±0.26	11.98±0.24	12.01±0.14	11.88±0.17	11.93±0.12
Ratio AL/EQD	Treated eye	0.805±0.02**	0.781±0.03*	0.797±0.02*	0.778±0.02	0.791±0.03*	0.795±0.08*	0.783±0.02
	Normal eye	0.765±0.01	0.771±0.01	0.778±0.02	0.769±0.01	0.760±0.01	0.770±0.01	0.766±0.01
Wet eye weight (g)	Treated eye	0.71±0.06**	0.65±0.03**	0.71±0.04***	0.68±0.03*	0.70±0.03**	0.67±0.02**	0.66±0.03
	Normal eye	0.61±0.04	0.60±0.03	0.62±0.03	0.63±0.03	0.64±0.03	0.61±0.02	0.62±0.02

*Differences between treated and normal eyes significant at *P < 0.05, **P < 0.01, ***P < 0.005, Wilcoxon matched-pairs signed-ranks test (one-tailed).*

APPENDIX III

IMMUNOCYTOCHEMISTRY OF NEUROTRANSMITTERS IN THE RETINA AND THE EFFECTS OF FORM-DEPRIVATION

AIII.1. Summary

Form-deprivation myopia has previously been linked with decreases in retinal dopamine levels in both chick and monkey, and increased VIP (but not substance P) in monkey retina. In this study, chicks were monocularly occluded from hatching for 10 days; immunolabelling for GABA, glycine, glutamate, glutamine and taurine was then carried out on central and peripheral parts of retinae from normal and myopic chicks. Normal retinas showed extensive labelling for all four putative neurotransmitters and the one metabolite. Photoreceptor inner and outer segments were immunoreactive for taurine; most outer segments also showed glutamatergic labelling. Horizontal cells displayed GABA immunoreactivity. GABAergic bipolar cells were located within the inner zone, and glycinergic bipolars, within the central zone of the innernuclear layer. A novel finding was that most of the bipolar cells also labelled strongly for taurine; this suggests that in chick, taurine may be a candidate bipolar cell transmitter. Most of the bipolar cells that contained taurine also contained glutamate, and were located at the outer aspect of the innernuclear layer. Most amacrine cells were strongly GABAergic, whilst a sub-population were immunoreactive for glycine. Layers within the innerplexiform layer were immunoreactive for all four putative transmitters, but GABA immunolabelling was predominant. Most ganglion cells were glutamatergic but sparse populations labelled for GABA, glycine, and taurine. Immunolabelling for GABA, glycine, glutamate, glutamine and taurine appear to be unaffected by form-deprivation.

AIII 2. Introduction

In the chick form-deprivation myopia primarily results from an expansion of the vitreous chamber, which also causes the retina to expand (Teakle *et al.*, 1993). The retinal expansion results in retinal thinning, but it is thought that the gross organization of the retina is unaffected (Yinon *et al.*, 1982/1983). It has been shown that the deprivation effect is a local ocular one and thus the retina must have a key role in the formation of an anomalous growth signal (reviewed in Wallman, 1993). As pattern vision is a major retinal function, neurotransmitters, neuropeptides and retinal metabolites may be affected by visual deprivation. Variations of these molecules may then influence eye growth.

Some retinal cells and transmitters that may be involved in this process have been studied. It seems unlikely that ganglion cells have a key role, as form-deprivation myopia still develops following the elimination of ganglion cells by optic nerve section (Troilo *et al.*, 1987; Wildsoet and Pettigrew, 1989). It has been suggested that amacrine cells are the most likely candidates (reviewed in Wallman, 1991). Neurotoxin studies support the hypothesis that amacrine cells are involved; kainic acid injections produce vitreous chamber enlargement (Wildsoet and Pettigrew, 1988; Barrington *et al.*, 1989) while quisqualic acid injections decrease vitreal growth and deepen the anterior chamber (Barrington *et al.*, 1989). The biochemical mechanism by which retinal responses are translated into signals that regulate eye growth is unknown. Researchers have linked form-deprivation myopia with decreases in retinal dopamine levels in both chick and monkey, and increased VIP (but not substance P) in monkey retina (reviewed in Laties and Stone, 1991). In addition, apomorphine injections have been shown to decrease the effects of deprivation in chick (Stone *et al.*, 1989). It has recently been shown that GABA may regulate both retinal dopamine and melatonin biosynthesis in chick retina, with the melatonin effect being indirect, i.e. due to inhibition of dopamine activity (Kazula *et al.*, 1993). The most abundant putative neurotransmitters in retina; GABA, glycine, glutamate and taurine and the metabolite glutamine have not previously been studied in relation to this condition. The morphological and immunocytochemical characteristics of the major cell types, in central and peripheral regions of retina from normal and myopic chick eyes

using antibodies to these major transmitters and metabolite was investigated.

AIII 3. Methods

Animals and ocular measurements

Male White Leghorn-New Hampshire cross chicks were obtained from a local hatchery on the day of hatching. They were raised in temperature controlled enclosures with food and water provided *ad libitum*. Chicks were exposed to a 12 hr/12 hr light-dark cycle, with lights on at 7 am and off at 7 pm and light intensity of 250 lux at the level of the food trough. Chicks were monocularly occluded from hatch, both right and left eyes were used for occlusion.

On day 10 the ocular parameters were measured. Chicks were anaesthetised using halothane and retinoscopy and A-scan ultrasonography (Wallman and Adams, 1987) performed under dim illumination to determine the refractive error and the positions of the intraocular surfaces respectively. Anterior chamber depth (ACD), axial lens thickness (ALT), vitreous chamber depth (VCD) and axial length (AL) data were obtained (see Appendix I for more details).

Tissue preparation

Chicks were overdosed with sodium pentobarbitone (60 mg/kg I.P.), the eyes excised, anterior segments removed and the posterior segments fixed with 2.5% glutaraldehyde for 30 min in 0.1 M cacodylate buffer, pH 7.2. Pieces of retina measuring 2.5 x 1.5 mm, from the superior retina and visual streak regions of each eye, were dehydrated in graded concentrations of acetone and embedded in Durcupan resin. Thermal polymerisation of the specimens was carried out at 70°C for 24 hr. Transverse, 1 μm-thick sections were then cut for light-microscopic immunolabelling for GABA, glycine, glutamate, glutamine and taurine.

Immunolabelling

Immunolabelling for GABA, glycine, glutamate, glutamine and taurine was carried out on the semithin sections as described by Pow and Crook (1993). Sections were placed onto multiwell, glass microscopic slides, air

dried, etched for 10 min using sodium ethoxide solution, washed with ethanol and distilled water. Sections were then incubated for 30 min in 1% BSA in phosphate buffered saline to prevent non-specific labelling. Sections were incubated overnight in the primary antibody, washed in PBS-BSA and then incubated in a biotinylated secondary antibody against rabbit immunoglobulins (Amersham) at a dilution of 1:300 for 3 hr. Sections were washed and then similarly incubated in HRP-streptavidin-biotin complex (Amersham). Sections were washed and DAB used as a chromagen to reveal the immunolabelling. The DAB reaction was further intensified with silver. Sections were viewed with Nomarski DIC optics using a Zeiss Axioplan light micropscope and the labelling of retina from treated and normal eyes compared. The specific rabbit polyclonal antibodies used here have been shown to be of extremely high titre and to be highly specific (Pow and Crook, 1993). Although sections from both peripheral and central regions were analyzed only photomicrographs of central retina are shown here. While three chicks were used for this study all of the photomicrographs are of retina from one animal.

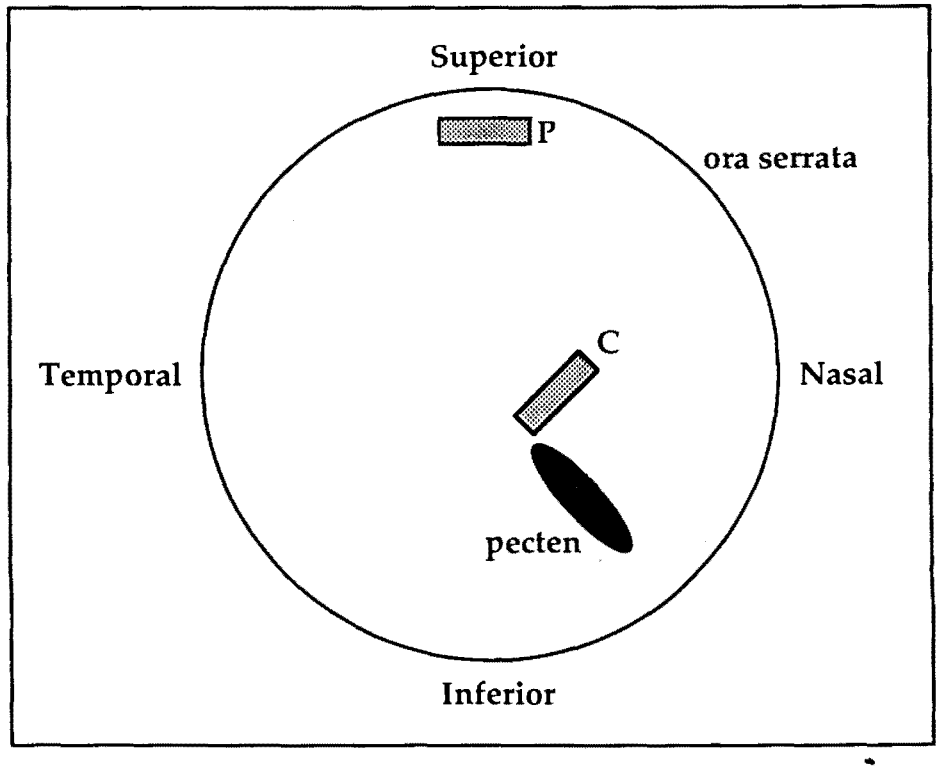


Figure AIII.1. The two retinal sampling sites (C) central and (P) peripheral are shown schematically for a right eye.

AIII 4. Results

Normal characteristics of immunolabelling

Normal retinas showed extensive labelling for all four putative neurotransmitters and single metabolite (Plates AIII.1, AIII.2, AIII.3, AIII.4, AIII.5). Photoreceptor inner and outer segments were immunoreactive for taurine; most outer segments also showed glutamatergic labelling. Horizontal cells displayed GABA immunoreactivity. GABAergic bipolar cells were located within the inner zone, and glycinergic bipolars, within the central zone of the INL. A novel finding was that most of the bipolar cells also labelled strongly for taurine; this suggests that in chick, taurine may be a candidate bipolar cell transmitter. Most of the bipolar cells that contained taurine also contained glutamate, and were located at the outer aspect of the INL. Most amacrine cells were strongly GABAergic, whilst a sub-population were immunoreactive for glycine. Layers within the innerplexiform layer were immunoreactive for all four putative transmitters, but GABA immunolabelling was predominant. Most ganglion cells were glutamatergic or glutaminergic but sparse populations labelled for GABA, glycine, and taurine. Glutaminergic immunolabelling of displaced ganglion cells and interplexiform cells was observed. The labelling of major cell types is summarized in Table AIII.1.

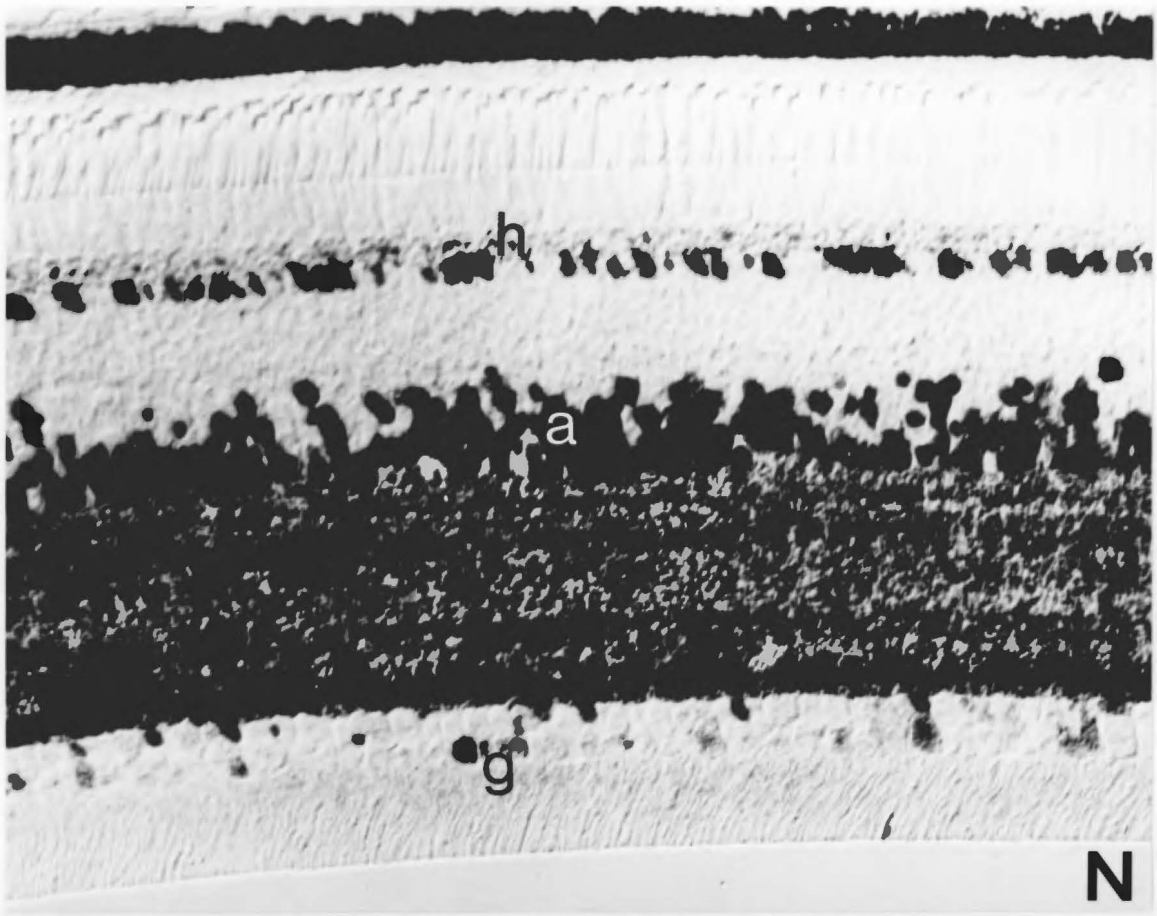
Table AIII.1. Summary of immunolabelling.

Cell type	GABA	Glycine	Glutamate	Glutamine	Taurine
Photoreceptor	no	no	outer segment	no	yes
Horizontal	yes	no	yes	no	no
Bipolar	no	scattered	yes	no	scattered
Amacrine	yes	yes	scattered	scattered	scattered
Ganglion	scattered	scattered	yes	yes	scattered

Plate AIII.1

(overleaf)

Plate AIII.1. Light micrographs of central retinal sections from **N.** a normal eye and **T.** from a myopic eye immunolabelled for GABA. The horizontal (**h**), bipolar (**b**) and amacrine (**a**) cells are strongly labelled. Synapses within the interplexiform layer are strongly GABAergic, as are scattered ganglion cells (**g**). The pattern of labelling is similar for retina from normal and myopic eyes. Scale bar equals 10μm.


b
a
g
N

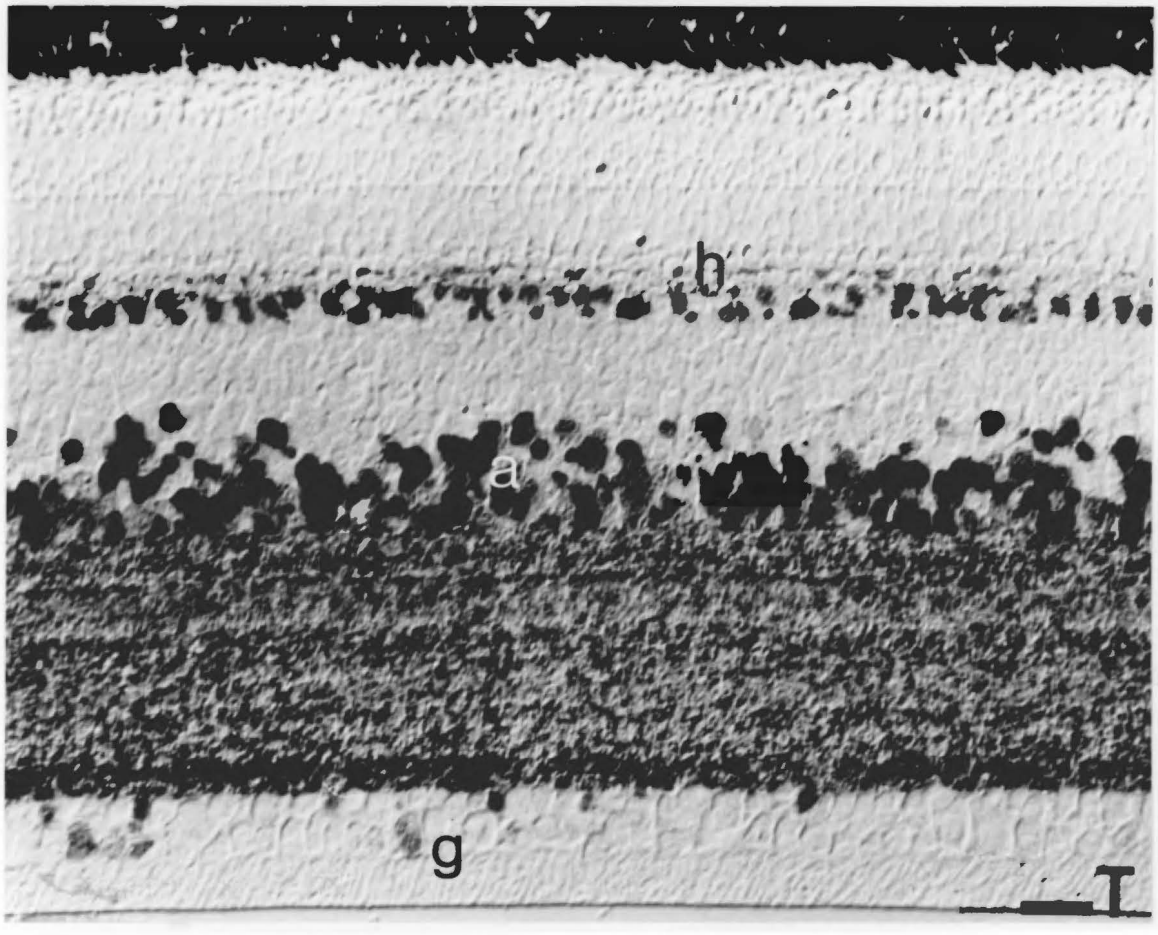

b
a
g
T

Plate AIII.2

(overleaf)

Plate AIII.2. Light micrographs of central retinal sections from **N.** a normal eye and **T.** from a myopic eye immunolabelled for glycine. The bipolar cells (**b**) in the central innernuclear layer are strongly labelled, there is a scattering of labelled ganglion cells (**g**) and a lack of labelling of photoreceptors (**p**). Synapses within the innerplexiform layer are strongly glycinergic. The pattern of labelling is similar for retina from normal and myopic eyes. Scale bar equals 10μm.

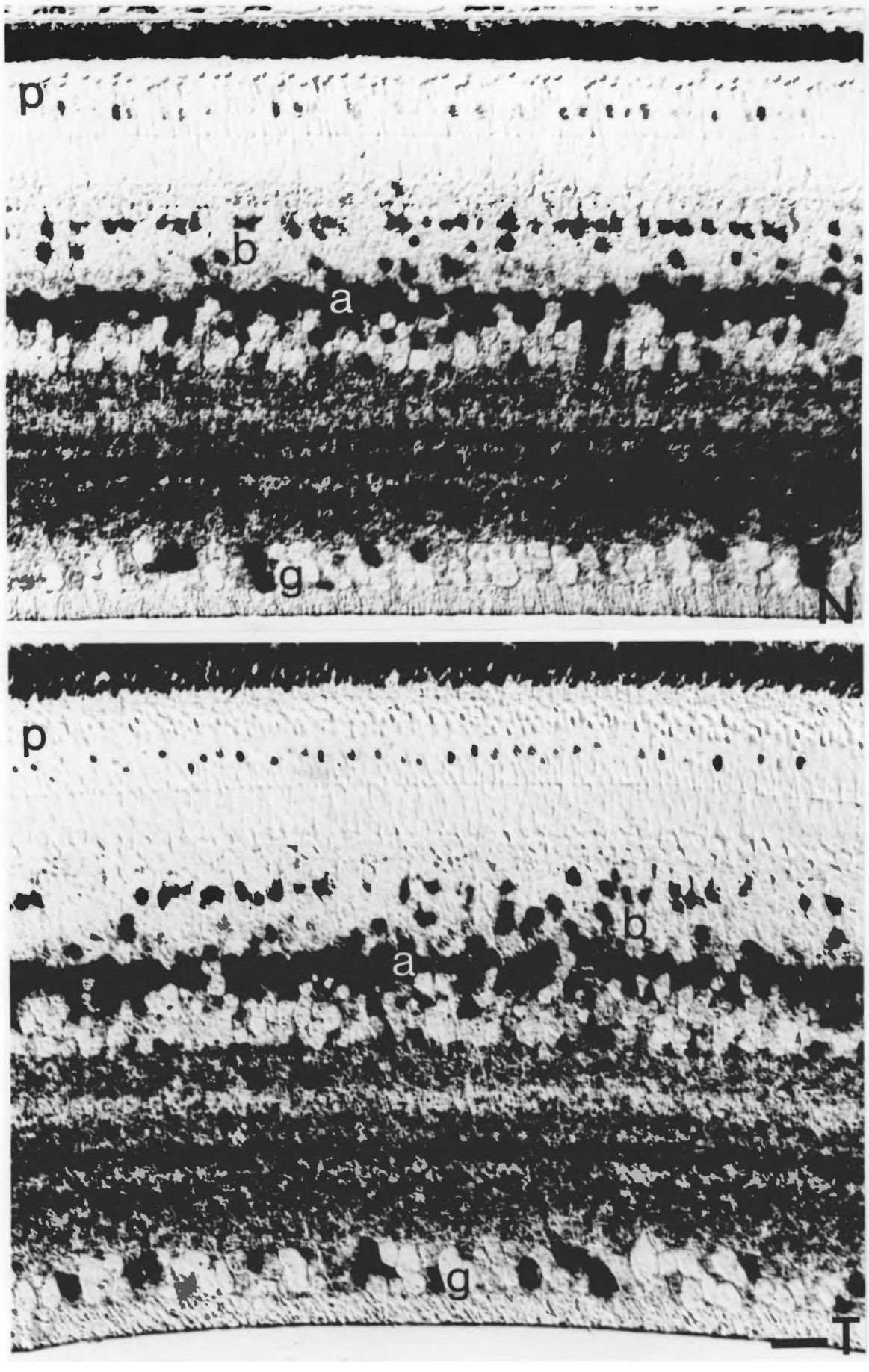

p
b
a
g
N
p
b
a
g
T

Plate AIII.3

(overleaf)

Plate AIII.3. Light micrographs of central retinal sections from **N.** a normal eye and **T.** from a myopic eye immunolabelled for glutamate. Most photoreceptor (**p**) outer segments show glutamate labelling, glutamatergic bipolar cells (**b**) are located at the outer aspect of the innernuclear layer and many ganglion cells (**g**) are glutamatergic. Synapses within the innerplexiform layer are strongly glutamatergic. The pattern of labelling is similar for retina from normal and myopic eyes. Scale bar equals 10μm.

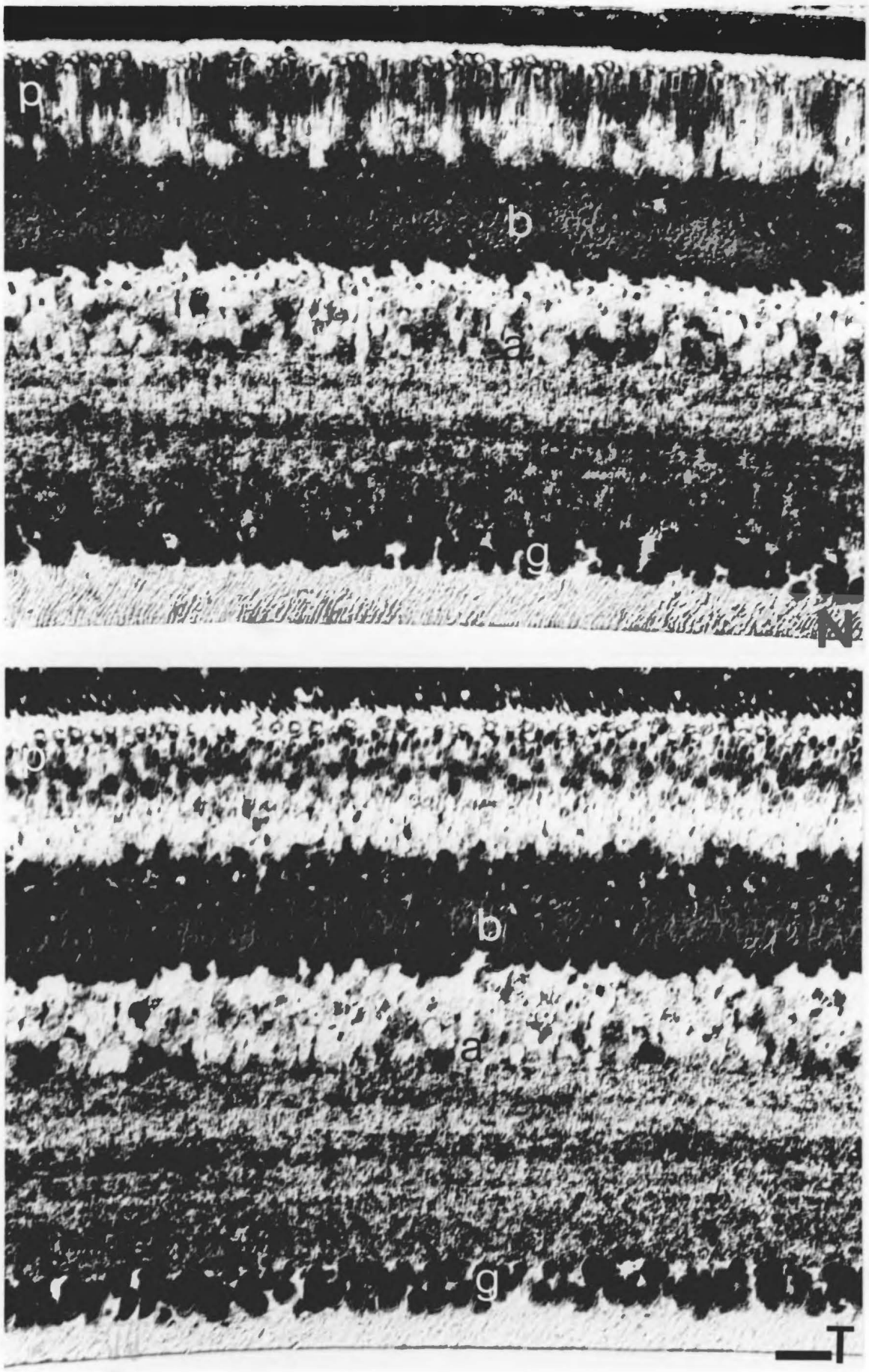

p
b
a
g
N
p
b
a
g
T

Plate AIII.4

(overleaf)

Plate AIII.4. Light micrographs of central retinal sections from **N.** a normal eye and **T.** from a myopic eye immunolabelled for glutamine. The ganglion cell layer is densely labelled. There are large displaced glutaminergic ganglion cells (**dg**) and glutaminergic interplexiform cells (**ip**). The pattern of labelling is similar for retina from normal and myopic eyes. Scale bar equals 10μm.

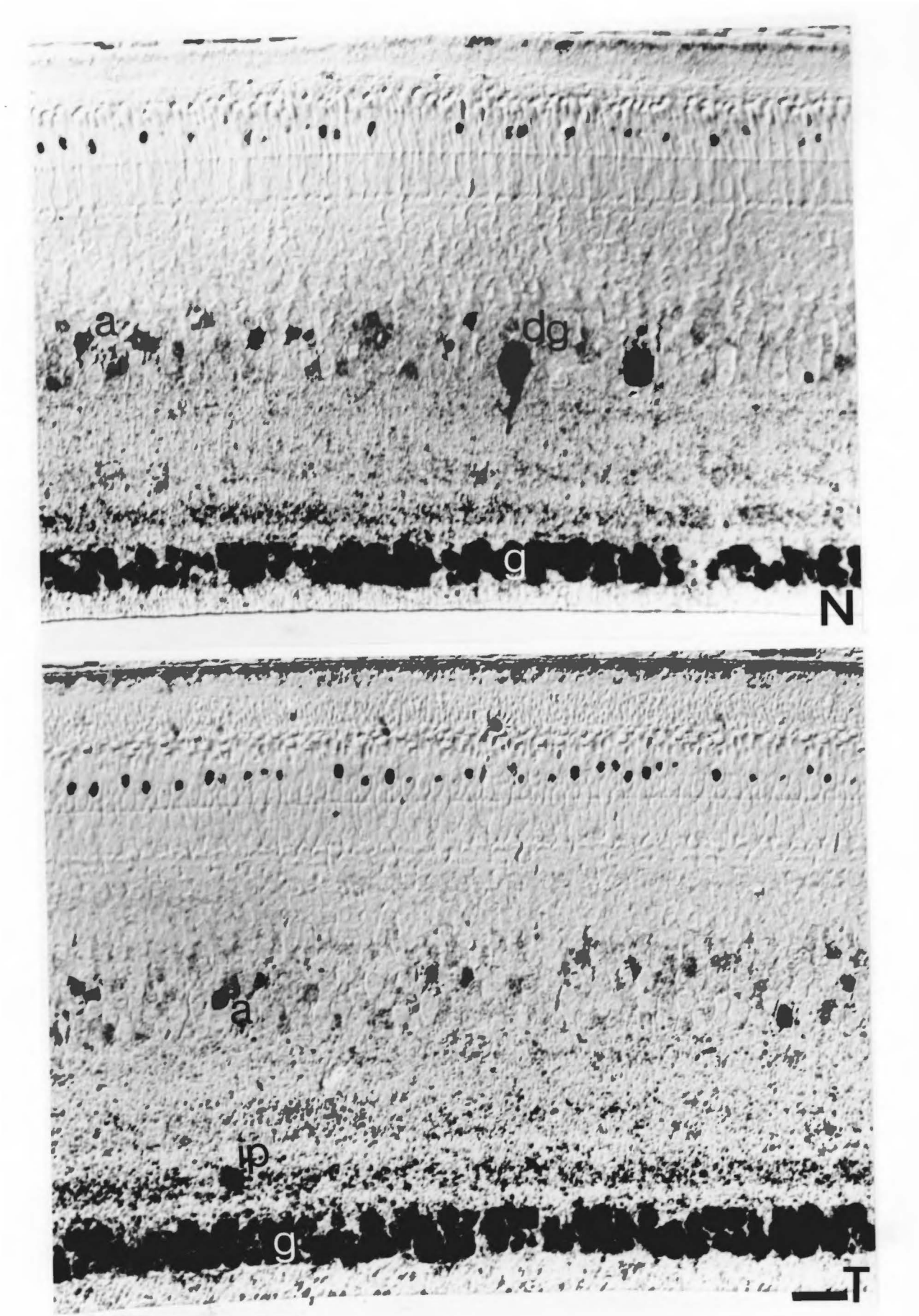
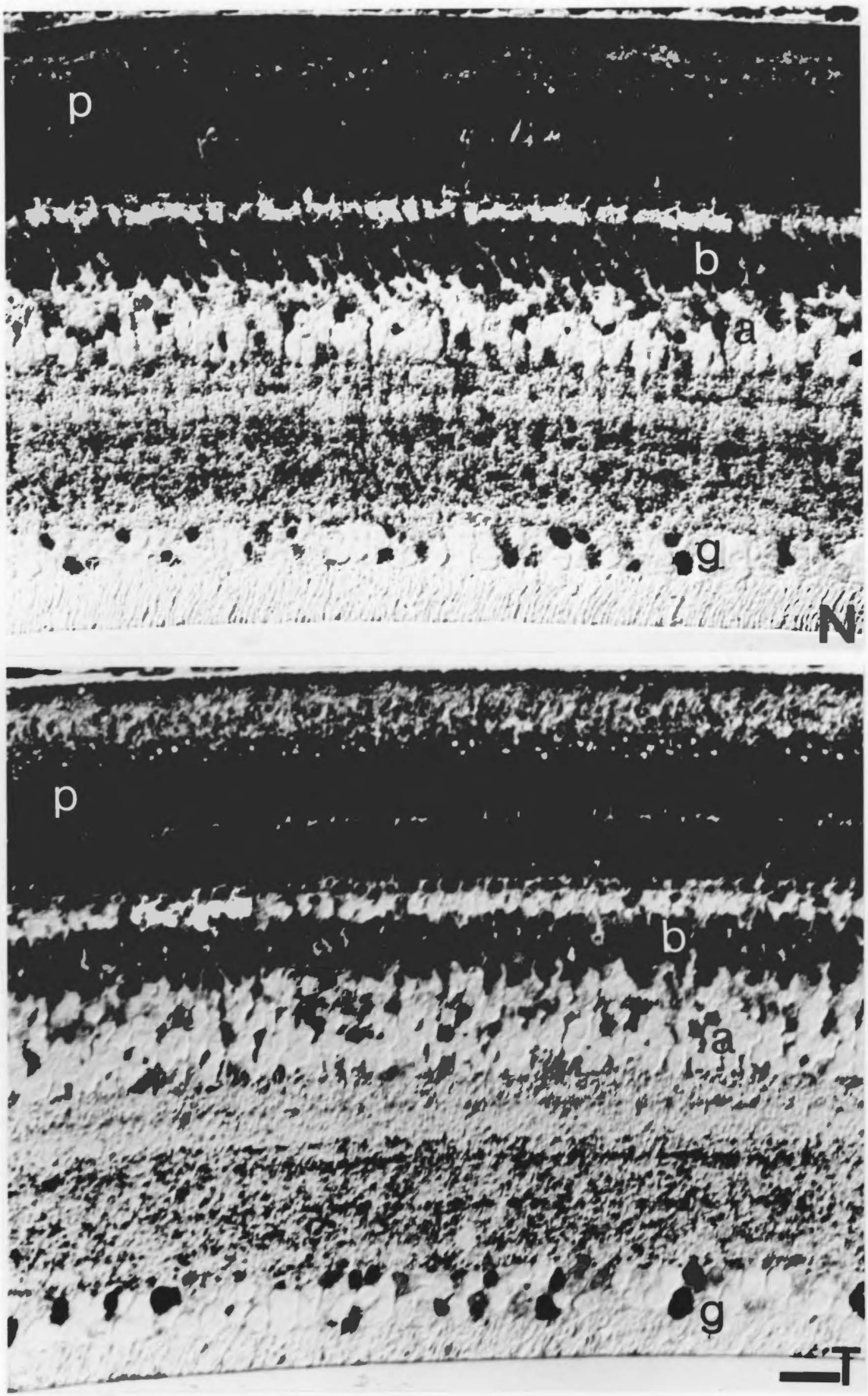

a
dg
g
N
a
ip
g
T

Plate AIII.5

(overleaf)

Plate AIII.5. Light micrographs of central retinal sections from **N.** a normal eye and **T.** from a myopic eye immunolabelled for taurine. The inner and outer segments of the photoreceptors are densely labelled, as are a large number of bipolar cells (**b**). Scattered ganglion cells (**g**) are taurinergic. The pattern of labelling is similar for retina from normal and myopic eyes. Scale bar equals 10μm.


p
b
a
g
N
p
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g
T

Effects of deprivation on eye growth and refraction

After 10 days of occlusion treated eyes were 1.1±0.3 mm longer axially and −24±7 D more myopic than normal eyes.

Effect of retinal expansion on immunolabelling

Form-deprivation resulted in vitreous expansion and high myopia. The histological examination of retina from myopic eyes did not reveal any qualitative differences compared to the retina from normal eyes. Intensity and location of immunolabelling was similar for both treated and normal eyes. Findings suggest that immunolabelling for GABA, glycine, glutamate, glutamine and taurine were unaffected by form deprivation.

AIII.5. Discussion

Normal retinal labelling

Supporting the results of Kalloniatis and Fletcher (1993) this study found that the "through" retinal elements, i.e. photoreceptors to bipolar cells to ganglion cells were immunoreactive for glutamate. This pattern of labelling was not seen with any of the other putative neurotransmitters studied. The lateral pathway, i.e. amacrine and horizontal cells, appears to be both glycinergic and GABAergic. Due to the many subclasses of major retinal cells types, all showed immunoreactivity to two or more of the studied transmitters.

Comparison to retina of myopic chick

No significant differences in the immunohistochemical reactivity for GABA, glycine, glutamate glutamine and taurine of retina from normal and myopic chicks were observed. Retina from treated and normal eyes showed similarity in the localization of all the putative neurotransmitters studied and on the whole there was similarity in the intensity of labelling. However, it is possible that very subtle changes were present that went undetected. The increased eye growth seen with form deprivation may be the result of very slight alterations in normal levels of any one of these transmitters or other molecules. Such slight changes may be below the threshold for discrimination with this

technique. It should also be mentioned that this technique cannot quantify the amount of transmitter present and can only be used at a gross level to indicate whether particular cells have a higher concentration or lower concentration of a particular transmitter.

It is reported that neurochemical differentiation of the avian retina is complete very soon after hatch (DeMello *et al.*, 1976) and except for slight retinal thinning, the retina from myopic appear normal, at least at a gross level (Yinon *et al.*, 1982/1983). It would thus be expected that very sensitive techniques would be required to detect any alterations in retinal physiology and neurochemistry that presumably occur with deprivation. However, using a very similar technique Laties and Stone (1991) observed increased VIP (but not substance P) in retina from myopic monkeys. The main difference to the technique used here was the use of immunofluorescence to visualize the labelling.

Although negative results were found here form-deprivation myopia has been linked to decreases in retinal dopamine levels in chick (reviewed in Laties, 1991). As eye growth is controlled locally within the eye (Wallman *et al.*, 1987) this suggests that the retina must increase or decrease the release of some substance or substances that then alters growth. Whilst only putative retinal neurotransmitters and a single metabolite were studied here growth factors may be involved in this process. Basic fibroblast growth factor has been implicated as one of the growth factors that may be involved in the control of eye growth (Rohrer *et al.*, 1993).

AIII.6. Conclusion

The findings suggest that immunohistochemical reactivity for GABA, glycine, glutamate, glutamine and taurine is unaffected, at least at a gross level, by form deprivation.

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